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A CLINICAL BIO-ASSAY FOR CHORIONIC GONADOTROPIN

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THE value of the bio-assay of chorionic gonadotropin in clinical endocrinology is well recognized. However, in spite of the numerous methods employed for the quantitative determination of chorionic gonadotropin in experimental work, there does not exist a satisfactory procedure for routine use in clinical laboratories, especially when the number of determinations is large. A bio-assay suitable for routine use in a clinical laboratory should have the following attributes: 1) it should be sufficiently simple to be performed by average laboratory technicians; 2) it should not require too much time for completion; 3) it should be expressed in terms of the International Standard; 4) the error of the assay should be known, and this should be of an order that would allow determinations accurate enough for clinical use.

The purpose of this paper is to describe the procedure that has been developed and in use in our laboratory for the past four years (Albert (1)). The method involves no new principles; rather, well-known reactions and methods were adapted to the function of a routine clinical laboratory. In contrast to older procedures which require concentration of the hormone by chemical or physical methods, serial dilution of the concentrate and

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multiple injections into animals, the method herein described utilizes a single injection of unconcentrated blood serum or urine. Older methods require days to weeks for completion, whereas this procedure is completed in four hours. Previous methods utilize animal units of one kind or another, a procedure which has only local significance and cannot be duplicated satisfactorily in other laboratories. The results of the method to be described are stated in terms of the international unit (I. U.), which is a standard of general reference and a unit of measurement universally understood, thus allowing a ready exchange of results between different workers and laboratories. Finally, in most methods for assay of this hormone the error of the method is unknown. The method to be described has been found to have an error of ± 31 per cent, which is sufficiently accurate for clinical purposes.

MATERIALS AND METHODS

Weanling female rats obtained from a highly inbred colony (Wistar strain) were used. The biologic end point for the detection of chorionic gonadotropin was hyperemia of the ovary. This hyperemia was seen many years ago by Zondek and associates (2), and through a long series of investigations has become the basis of a routine qualitative method for the laboratory diagnosis of pregnancy (3, 4, 5). It was chosen as the end point for quantitative work because of the rapidity of the response and because of the simplicity and convenience with which it can be used.

Preliminary investigation was carried out to define the optimal weight and age of rats to be used. It was found that rats ranging in age from 22 to 26 days and in weight from 35 to 50 Gm. gave fairly uniform responses to known amounts of chorionic gonadotropin. Rats weighing more than 50 Gm. were less responsive, and rats weighing less than 35 Gm. gave greater response to 1.0 I.U. of hormone.

For convenience, the volume of injection was always 1.0 cc. and the site always subcutaneous. The rats were killed by ether asphyxiation, the abdomens were opened, and the ovaries were removed by wide dissection and placed on wet blotting paper. With examination under an ordinary desk lamp, it is a simple matter to distinguish hyperemic from nonhyperemic ovaries; and with some practice, any laboratory technician can read the results satisfactorily.

To establish standards, the International Standard preparation was dissolved in saline solution to obtain any needed concentration. Recovery experiments were performed with both the International Standard preparation and with certain commercial preparations: Korotrin, Winthrop-Stearns, Inc., New York; and Follutein, E. R. Squibb & Sons, New York; to which firms we are indebted for generous supplies of their products.

RESULTS

1. Time-response curves.—Large numbers of rats were injected with various amounts of chorionic gonadotropin and groups of animals were killed at various intervals after injection. The purpose of these experiments was to find the most appropriate time at which the readings should be made. Doses ranging from 0.5 I.U. to 300 I.U. were used; groups of rats were killed at hourly intervals for six hours, and the percentage of hyperemic ovaries was determined. These results are shown in Figure 1, in which the per-

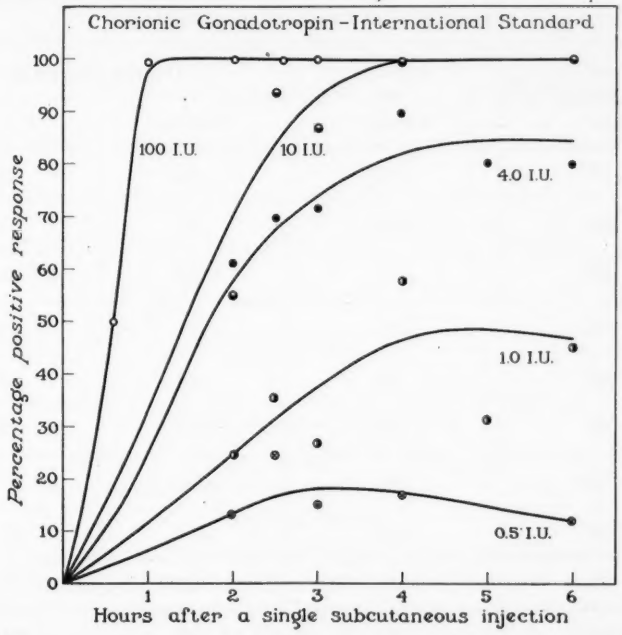

FIG. 1. The response (hyperemia) of the ovaries of rats at various intervals following the injection of chorionic gonadotropin (International Standard). Doses between 0.5 I.U. per rat and 100 I.U. per rat are shown. Each point represents 20 to 40 ovaries.

centage positive response (hyperemia) is plotted against time in hours following injection, and this for several doses of hormone. Each point represents the average result in from 20 to 40 ovaries. The greater the amount of hormone administered, the sooner a response of given magnitude occurred; and for any given time, the greater the dose, the greater was the percentage of ovaries affected. More important to observe, however, is that at four hours following injection, the response became asymptotic at all dose levels. Although it would be possible to construct a scheme of assay

planned for observation of response at one hour following injection, rather large amounts of hormone would be required. The fact that the percentage response was stabilized at about four hours for both high and low doses, as well as the greater sensitivity at this time than earlier, served to set four hours as the interval of time following injection at which readings should be made.

2. Dose-response reaction.—Groups of rats were injected with doses of chorionic gonadotropin ranging between 0.25 I.U. and 5 I.U., and observations were made of the percentage of ovaries found hyperemic four hours following injection. These experiments were performed at various intervals over a period of two years, each experiment involving two to five doses and utilizing as many animals as were available at any time. There were twenty-two such experiments in which a total of 1,079 rats were used. The first twelve experiments were performed within a period of three months, and the remaining ten experiments were performed over the ensuing two years. The purpose of this series of experiments was to determine, first, whether the dose-response relationship was consistently reproducible and, second, whether it remained so over a long interval of time.

TABLE 1. SUMMARY OF EXPERIMENTS WITH THE INTERNATIONAL STANDARD PREPARATION OF CHORIONIC GONADOTROPIN

Dose, international units	Number of ovaries	Ovaries hyperemic	
		Number	Per cent
0.25	102	13	12.7
0.33	360	46	12.8
0.50	248	66	26.6
1.00	586	271	46.2
1.50	44	35	79.5
2.00	170	129	75.9
3.00	360	295	81.9
4.00	172	158	91.9
4.50	44	39	88.6
5.00	72	72	100.0

To each of the twenty-two experiments a logistic curve (6, 7) was fitted by plotting the logits of the percentage response against the logarithm of the dose and fitting a straight line to the points by least squares, determining in each case the slope b and the dosage of hormone effective in inducing a response in half of the ovaries (E.D. 50). The values of the slope constants b and E.D. 50's were not materially different, one from the other, in the twenty-two separate experiments, indicating that large periodic changes do not occur. Consequently, the observations were pooled (Table 1), and

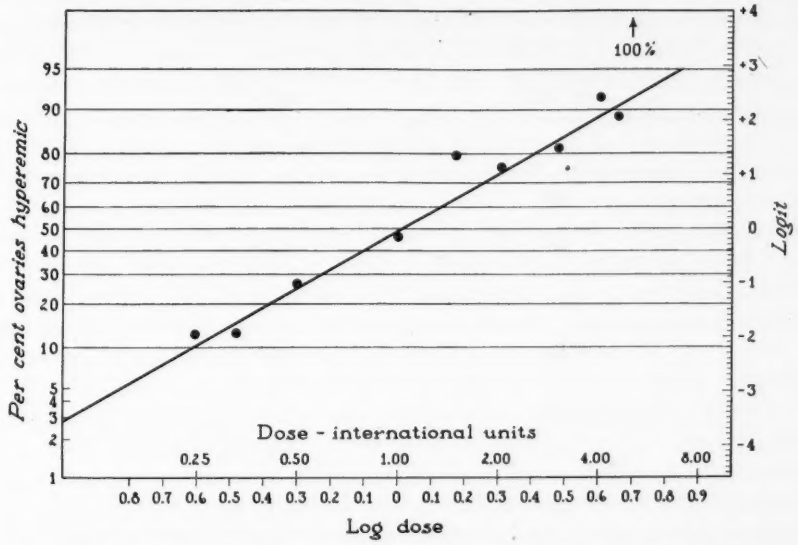

FIG. 2. Fit of logistic line to the pooled data in Table 1. The logit of the percentage response was plotted against the logarithm of the dose (or the percentage directly against dose on log-logistic ruled paper, Codex Company, Norwood, Massachusetts), and a line was fitted by least squares, yielding the equation $l=3.52x-0.0729$, where l is the logit of the percentage hyperemic and x is the log dose. This fitted line furnished the values in I.U. corresponding to percentage of ovaries hyperemic (Table 2).

a logistic line was fitted to the data of the entire experience (Fig. 2). From this line were calculated the units of gonadotropin corresponding to an observed percentage of ovaries affected, and these are given in Table 2.

3. Determination of the error of assay.—An empiric test of the accuracy of the method was obtained by performing recovery experiments; that is, known amounts of the International Standard were tested by the laboratory personnel as unknowns. A dilution set of three doses of chorionic gonadotropin differing by about 0.5 log unit was prepared, each of the doses was injected into 5 rats, and the percentage of total ovaries showing

positive response was determined. The dose in I.U. corresponding to the percentage response was then read from Table 2. These values were adjusted according to the dilution factor, and the three adjusted values were averaged, giving the mean concentration in I.U. per cubic centimeter of the unknown. This calculated value was compared with the known value, and the deviation from the known value was expressed in percentage.

An example of this procedure follows: The International Standard was dissolved in saline solution to yield a concentration of 4.5 I.U. per cubic

TABLE 2. INTERNATIONAL UNITS CORRESPONDING TO PERCENTAGE RESPONSE

Response, per cent	International units
10	0.25
20	0.42
30	0.60
40	0.80
50	1.05
60	1.37
70	1.83
80	2.60
90	4.41

centimeter. From this solution (A), two dilutions were made: 1.5 I.U. per cubic centimeter (B), and 0.5 I.U. per cubic centimeter (C). A, B and C were injected, and the proportion of ovaries which were hyperemic was recorded. The results for A, B and C were 90, 60 and 20 per cent respectively. From Table 2, these percentages correspond respectively to doses of 4.41, 1.37 and 0.42 I.U. Adjusted for dilution, the respective values become 4.41 I.U. $\times 1 = 4.41$ I.U. per cubic centimeter; $1.37 \times 3 = 4.11$ I.U. per cubic centimeter; and 0.42 I.U. $\times 9 = 3.78$ I.U. per cubic centimeter. The mean of these values is 4.1 I.U. per cubic centimeter. Compared with the actual concentration of 4.5 I.U. per cubic centimeter, the estimated value is in error by -9 per cent. Sixty-six such tests, employing a total of 990 rats, were made; and for these the mean error was -4 per cent and the standard deviation of the errors was 31 per cent, or a probable error of 21 per cent. This means

that in 50 per cent of the tests, the error can be expected to be within about ±21 per cent, and in 67 per cent of the cases within about ±31 per cent.

4. Application of the assay to clinical material.—The preceding experiments indicated that chorionic gonadotropin could be measured with reasonable accuracy with 15 animals (three doses, each given to 5 animals) by means of a standard curve. Such determinations were made on purified hormone. It was necessary, however, to ascertain whether similar results could be obtained under clinical conditions; that is, in blood and urine.

Accordingly, known amounts of the International Standard were added to serum or urine of either male or female persons not producing chorionic gonadotropin, and the artificial mixtures so prepared were assayed. Since the large amount of serum protein could affect the assay in any number of ways, the first experiment consisted of making a series of dilutions of serum with saline solution, varying from 100 per cent to 0 per cent serum, each dilution containing 1.0 I.U. of hormone per cubic centimeter. Each dilution was then tested on 18 rats. The results of this one-dose experiment showed that the 0 per cent serum (saline control) and the 10 per cent serum yielded the expected response, whereas there was a somewhat smaller response for the 15 per cent, 20 per cent and 25 per cent serum. From 25 per cent to 100 per cent serum, the values fell sharply, the response obtained being equivalent to only one third of the hormone added.

This diminution in response was avoided by diluting serum 1:10 for assay, since the 10 per cent serum yielded the same response as saline control. To assure that valid assays can be obtained in 10 per cent serum solutions, serum containing no chorionic gonadotropin was diluted 1:10, yielding a 10 per cent serum dilution and to this, the International Standard gonadotropin was added to give varying concentrations of the gonadotropin, usually 3.0 I.U. per cubic centimeter, 1.0 I.U. per cubic centimeter and 0.3 I.U. per cubic centimeter. In some cases the 10 per cent serum solution containing 3.0 I.U. per cubic centimeter was further diluted, yielding 3 and 1 per cent serum solutions containing correspondingly less hormone concentrations. There were seven experiments of three doses each. The mean percentage error was +5 per cent, and the standard deviation was 27 per cent, showing that the gonadotropin could be assayed with as small an error in serum as in saline solution.

Similar experiments were carried out with the International Standard dissolved in pooled urine of normal persons of both sexes. Eight experiments were performed, and the results obtained were similar to those reported with serum solution of 10 per cent or less. The standard deviation of the error for the pooled data of these experiments was 30 per cent. Similarly, when a solution of urine containing 3.0 I.U. per cubic centimeter was diluted with saline solution to yield simultaneously both lower unitage and

less concentrated urine solutions, the assay results were not adversely affected.

The foregoing experiments indicate that ordinary constituents of urine have no effect on the assay, and likewise that constituents of serum have no effect on the assay provided the serum concentration is 10 per cent or less. These experiments also suggest that any synergism in response between chorionic and pituitary gonadotropin would be minimal in the dilutions used. Whether this holds also during pregnancy cannot be determined experimentally, since neither of the two direct ways of solving such a problem could be used: first, blood or urine of pregnancy could not be prepared in such a way that known amounts of chorionic gonadotropin could be added *after* the chorionic gonadotropin normally present had been selectively removed or destroyed without alteration of the remaining materials; and second, serum of nonpregnant persons could not be fortified with all the hormonal and other substances in amounts equal to those found in pregnancy and the resulting mixture tested by *adding* known doses of chorionic gonadotropin.

A few experiments were performed in order to test the stability of the hormone in serum or urine, since situations arise in which assays cannot be performed immediately. Sufficient International Standard was added to serum and urine to give a concentration of 3 I.U. per cubic centimeter of serum or urine. The mixtures were kept in the refrigerator and assayed daily. The response obtained was the same for two weeks in the case of serum and for one week in the case of urine, indicating a remarkable stability of the hormone under these conditions.

5. Method for clinical assays.—On the basis of the preceding experiments, the procedure for the clinical assay of chorionic gonadotropin in blood and urine was standardized. Urine or serum was diluted so that three dilutions, differing by about 0.5 log unit, were injected initially into 5 rats per dilution. This covered a tenfold range in amount of hormone; if necessary, as described later, a second, or a second and a third, series of three dilutions each would cover a hundredfold and a thousandfold range of hormone concentration. Ordinarily, the first three dilutions are all that is necessary, and nine dilutions, entailing the use of 45 rats, will suffice for most clinical situations.

Serum was diluted with saline solution so that three dilutions—1:10, 1:30 and 1:100—were obtained. One cubic centimeter of each of the dilutions was injected into each of a group of 5 rats, 15 animals in all being used for the three dilutions. The percentage of positive response was determined four hours after injection, and the calculation previously described was made. No series was employed unless it contained values greater than 50 per cent and other values less than 50 per cent. If all the responses were

greater than 50 per cent, further dilutions were made—1:100, 1:300 and 1:1,000—until the results obtained fell into the range of the standard curve. In the case of urine, the first series of dilutions consisted of undiluted urine, a 1:3 and a 1:10 dilution.

Example 1.—Five cubic centimeters of blood was obtained from a normal pregnant patient fifty-four days after onset of the last menses. The calculations are shown in Table 3.

TABLE 3. CALCULATION OF CONCENTRATION OF CHORIONIC GONADOTROPIN IN SERUM

Dilutions	Response, per cent of ovaries	I.U. (from Table 2)	Dilution factor	I.U./cc. serum
A. 2 cc. serum+18 cc. saline	70	1.83	10	18.3
B. 3 cc. A+6 cc. saline	40	0.80	30	24.0
C. 1 cc. A+9 cc. saline	10	0.25	100	25.0
				Mean = 22.4

Example 2.—A twenty-four-hour urine specimen was sent to the laboratory after a retroperitoneal mass in a man aged 28 years had been diagnosed on biopsy as chorio-epithelioma, grade 4. The calculations are shown in Table 4.

6. Specificity and sensitivity of the method.—The hyperemic reaction is not specific for chorionic gonadotropin, since pituitary gonadotropins, particularly luteinizing hormone and luteotropic hormone, also induce ovarian hyperemia. However, the lack of absolute specificity would be of no moment if it could be shown that the same amounts of urine or blood obtained from patients in whom chorionic gonadotropin was not present would yield negative responses. In effect, this would indicate that the foregoing pituitary hormones were not present in 0.1 cc. of serum or in 2 cc. of urine in amounts sufficient to induce positive ovarian responses. To ascertain this point, 200 specimens of urine were obtained through the courtesy of Dr. Stilwell, of the Division of Clinical Laboratories of the Mayo Clinic. Two cubic centimeters of each specimen was injected into each of 3 to 4 rats. None of the urines induced hyperemia, and study of the records of the patients disclosed no instance in which a diagnosis associated with the presence of chorionic gonadotropin could be made. Blood serum of 10 normal persons (nonpregnant women and a few men) was tested in a dose of 0.1 cc. per rat, and no positive responses were induced. Consequently,

the hyperemic reaction can be considered specific for chorionic gonadotropin under the stated conditions and with amounts of serum or urine used; namely, 0.1 cc. of serum or less and 2 cc. of urine or less.

These results, however, are in contrast with those of Farris (8), who employs the hyperemia test as a method for detection of ovulation, as reflected by the increased urinary excretion of pituitary gonadotropin at ovulation. The result of the test is also said to be positive in men who have recently experienced, or are anticipating, intercourse. Because such results

TABLE 4. CALCULATION OF CONCENTRATION OF CHORIONIC GONADOTROPIN IN URINE

Dilutions	Response, per cent of ovaries	I.U. (from Table 2)	Dilution factor	I.U./cc. serum
A. Undiluted urine	90	—	1	—
B. 3 cc. A+6 cc. saline	80	—	3	—
C. 1 cc. A+9 cc. saline	100	—	10	—
D. 1 cc. A+29 cc. saline	100	—	30	—
E. 1 cc. A+99 cc. saline	70	1.83	100	183
F. 1 cc. A+299 cc. saline	50	1.05	300	315
G. 1 cc. A+999 cc. saline	0	—	1,000	—

Mean = 249

Volume of urine in 24-hour sample = 1,060 cc.
Total 24-hour excretion of hormone = 1,060 × 249 = 264,000 I.U.

would seriously handicap the proposed method as an assay of chorionic gonadotropin, an experiment was performed utilizing both the hyperemia test as herein described (1 cc. of urine per rat) and a routine test (Frank, Salmon and Friedman, (9)) for pituitary gonadotropin. Both tests were performed on the same specimen of urine collected daily for an entire cycle in 2 normal menstruating women. Although the ovulatory peak in pituitary gonadotropin was obtained in both individuals, the results of all of the hyperemia tests were negative.

When a biologic test is employed to detect the presence of a hormone under conditions (blood or urine) in which other hormones capable of evoking the same reaction are present, the specificity of the biologic test

depends on its sensitivity to the hormone concerned and to the amount of the extraneous hormones. It may be possible in such a situation to adjust the design of the assay to achieve a high degree of specificity at the expense of sensitivity, or vice versa. In this assay, we have chosen to attain a high degree of specificity because 1) in those clinical situations in which chorionic gonadotropin is produced, there is usually a high concentration and therefore an easily measurable titer, and 2) assays for small amounts of hormone would require concentration of the blood or urine, and the results obtained might be due to pituitary gonadotropin alone if no chorionic gonadotropin were present.

For these two reasons, the assay was designed so that its extinction point (the smallest amount of chorionic gonadotropin that can be reliably measured) was 300 I.U. per twenty-four-hour specimen of urine and 3 I.U. per cubic centimeter of serum. This was arrived at by the following calculation. The minimal hyperemic response in a group of 5 animals that is considered reliable is 10 per cent. This value (Table 2) corresponds to 0.25 I.U. Assuming an average twenty-four-hour urine volume to be 1,200 cc., the minimal amount of hormone that can be measured when 1 cc. of urine is injected is, therefore, 0.25 I.U.$\times$1,200 or 300 I.U. per twenty-four hours. A similar calculation for serum gives 3 I.U. per cubic centimeter of serum. The failure of hyperemia to occur in tests of urine obtained during the ovulatory peak of gonadotropin in a normal menstrual cycle represents lack of response to 40 rat units per twenty-four hours of pituitary gonadotropin (9) or to an equivalent of 30 cc. of urine (1,200/40). Since 1 cc. of urine was injected, then a thirtieth of a rat unit of pituitary gonadotropin was ineffective in inducing hyperemia. However, if the assay of chorionic gonadotropin were to be made more sensitive so that the extinction point was, for example, 30 I.U. per twenty-four-hour sample, then 10 cc. of urine would be required for each rat. The possibility that the results obtained from a concentrate equivalent to 10 cc. of urine per rat might be due to pituitary gonadotropin is greatly increased, since now each rat receives 1/30$\times$10 or one third of a rat unit. Thus, by limiting the sensitivity of the method to about 300 I.U. per day, in effect "diluting out" pituitary gonadotropin, a high degree of specificity is achieved.

7. Comparison with values obtained by other methods.—A final appraisal of the proposed method is obtainable by comparing the values of the blood levels and urinary excretion of chorionic gonadotropin obtained by this method with those obtained by other quite different methods of assay. A search through the literature disclosed only two previous reports in which the assay of the hormone was given in terms of international units. The first of these, by Jones, Delfs and Stran (10), dealt with the concentration of hormone in the serum throughout normal pregnancy. The method of

assay employed consisted in extraction of serum, multiple injections over a three-day period and the use of increases in uterine weight of the rat as end point. The second study was made by Loraine (11) on the urinary excretion of hormone throughout normal pregnancy. The method of assay consisted of multiple injections into male rats and the use of increases in the weight of the seminal vesicles as the end point. Also, the older observations of Smith and Smith (12), which are given in rat units, have been converted

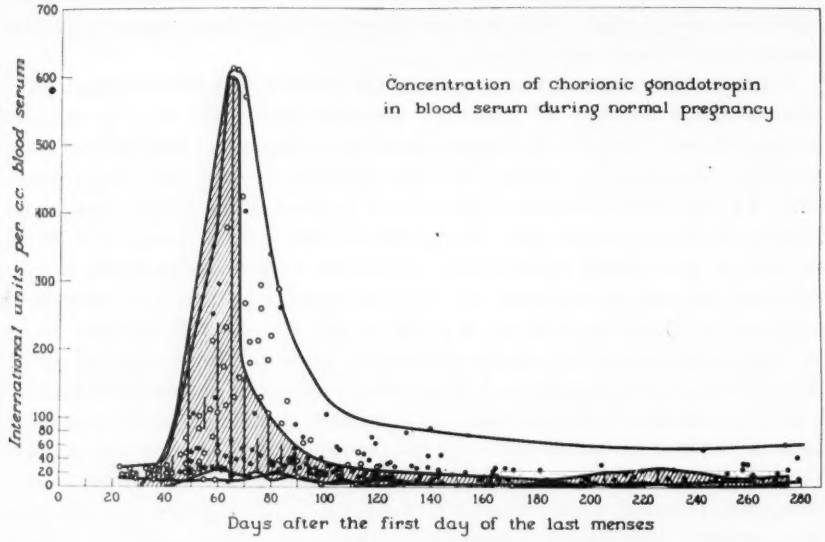

FIG. 3. Comparison of range of values of chorionic gonadotropin in the serum during human pregnancy. The hatched area represents range of values obtained in 24 patients by Jones, Delfs and Stran. The remaining 257 observations (open and closed circles) on 137 patients were obtained in our laboratory. They consist of 73 determinations on 6 patients during very early pregnancy (Smith, Albert and Randall), 20 observations on 4 patients (Gastineau, Albert and Randall), 112 determinations on 112 patients (Wilson, Albert and Randall) and 52 determinations on 15 patients not previously reported.

into international units by Rubin, Dorfman and Miller (13) and will be used for comparative purposes.

Figure 3 shows a comparison of the values obtained by Jones, Delf and Stran with those obtained in our laboratory. The data of Jones and associates consist of 187 determinations on 24 normal pregnant women, and the range of values is shown by the hatched area. The data obtained in this laboratory consist of 257 determinations on 137 patients and are included by the unshaded curve which limits the upper range of values. It can be seen that there is very good agreement between the two sets of data, with

the exception that we have obtained a somewhat wider range of values than Jones and associates obtained. Figure 4 compares our average values obtained during the last half of pregnancy with those of two other laboratories. In the shaded area to the right of Figure 4, the disappearance of chorionic gonadotropin from the blood following delivery of the placenta (48 determinations on 15 patients, from data of Johnson, Albert and Wilson (14)) is shown.

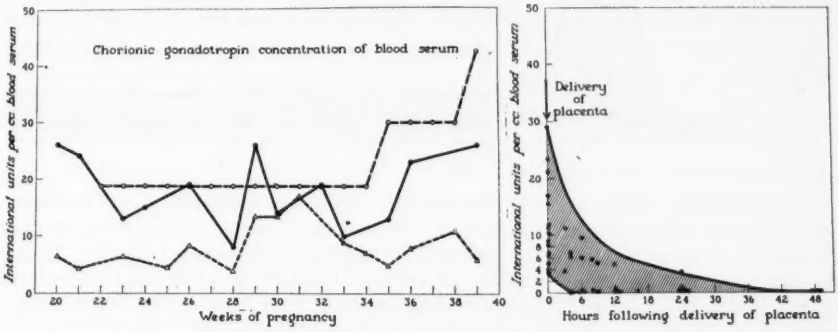
Chorionic gonadotropin concentration of blood serum
International units per cc blood serum
Weeks of pregnancy
Delivery of placenta
Hours following delivery of placenta

FIG. 4. Range of values of chorionic gonadotropin in the serum during the last half of pregnancy. Values are given as the median of the ranges found. Open circles are the data of Smith and Smith; closed circles are the values reported from our laboratory; triangles are the values of Jones, Delfs and Stran. At the extreme right are the values of Johnson, Albert and Wilson for the behavior of the hormone in the blood serum following delivery of the placenta (48 determinations on 15 patients).

A combined view of all of the data so far amassed in normal pregnancy leads to the following conclusion. Chorionic gonadotropin appears in the serum as early as the twenty-fourth day of a fertile cycle. Its concentration ranges from 3 to 30 I.U. per cubic centimeter until the fortieth day or so, when a sharp increase occurs. The peak value is slightly greater than 600 I.U. per cubic centimeter at about the sixtieth day after the first day of the last menses. The elevated phase lasts from the forty-fifth to the eighty-fifth day. After this time, the concentration is fairly uniform between 5 and 35 I.U. per cubic centimeter of serum until parturition. No apparently significant elevation prior to parturition, as described by Smith and Smith, was noted either in our data or in those of Jones, Delfs and Stran. Following delivery of the placenta, the hormone disappears from the serum within forty-eight hours.

The data on the urinary excretion of chorionic gonadotropin are given in Figures 5 and 6. In Figure 5 is shown the shape of the urinary curve for the entire period of gestation as a curve embracing all of the determinations made in our laboratory: 20 determinations on 4 patients (Gastineau, Albert

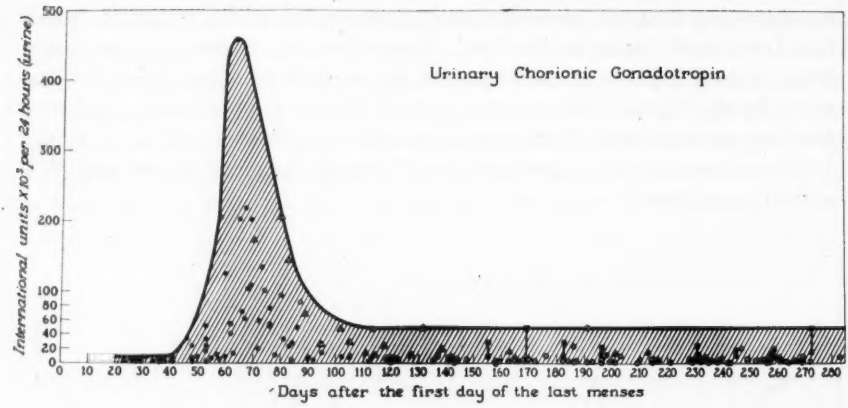
Urinary Chorionic Gonadotropin
International units x10³ per 24 hours (urine)
Days after the first day of the last menses

FIG. 5. Range of values for the urinary excretion of chorionic gonadotropin. The values shown by the individual symbols represent 178 determinations on 25 patients from this laboratory (20 determinations on 4 patients by Gastineau, Albert and Randall; 68 determinations on 6 patients by Smith, Albert and Randall; 90 determinations on 15 patients currently reported). The symbols connected by lines represent the observations of Smith and Smith.

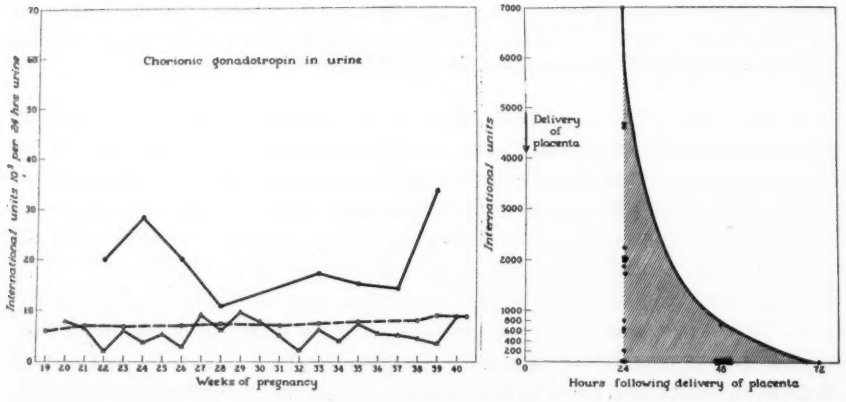
Chorionic gonadotropin in urine
International units 10³ per 24 hrs urine
Weeks of pregnancy
International units
Delivery of placenta
Hours following delivery of placenta

FIG. 6. Values of the urinary excretion of chorionic gonadotropin in the last half of pregnancy. Closed circles are the medians of the range of determination of Smith and Smith. Open circles are the average values of Loraine (92 determinations on 14 patients), and the open triangles are the median values of the observations reported by us (110 determinations on 19 patients). At the right are the values depicting the disappearance of the hormone from the urine following delivery of the placenta—47 determinations on 15 patients (Johnson, Albert and Wilson).

and Randall (15)), 68 determinations on 6 patients (Smith, Albert and Randall (16)) and 90 determinations on 15 patients currently reported. For comparison, the data of Smith and Smith (12) between 150 and 280 days of pregnancy are shown. The urinary curve is seen to be a reflection of the blood curve—an early constant and low level between the twenty-fourth and the fortieth day of about 5,000 I.U. per twenty-four hours, followed by an elevated phase with a peak value of nearly 500,000 I.U. per twenty-four hours at the sixtieth to the seventieth day. The range of duration of this elevated phase is between the fortieth and the ninetieth day. Until the end of pregnancy the excretion remains again at a constant level of about 8,000 I.U. per twenty-four hours. Figure 6 shows a comparison of average values during the last half of pregnancy. Our data agree more closely with those of Loraine than with those of Smith and Smith. On the right of Figure 6, the disappearance of the hormone from the urine after delivery of the placenta is shown. Within three days, the hormone can no longer be detected.

In both the serum and urine, the results obtained by the proposed method described in this paper are, therefore, in good agreement with those obtained by previous, quite different methods of assay.

SUMMARY

A method for the assay of chorionic gonadotropin in human blood and urine is described. The method utilizes ovarian hyperemia of the immature rat as the end point, the percentage response being proportional to the dosage over a range of 0.25 to 5 international units. The method is rapid, convenient and relatively highly specific, and has a standard error of ± 31 per cent. Comparisons with other methods indicate good agreement in values for the concentration of the hormone in blood and urine during normal human gestation. A comparative summary of normal values is given.

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THE EFFECT OF INTRAVENOUS ESTROGEN IN UTERINE BLEEDING*

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ONLY in recent years has an understanding of the physiology of functional uterine bleeding afforded a rational approach to successful therapy. Not until twenty-three years ago, when Allen (1) postulated that menstruation was due to cyclic reduction of the amount of estrogenic hormone in the body, was a scientific approach to the therapy of menorrhagia unveiled. Five years later the application of this work to the physiology of menstruation was modified by the observation of Smith and Engle (2) that estrogen withdrawal bleeding does not occur if withdrawal of this steroid is followed by injections of progesterone.

Since our present knowledge indicates that menstruation is directly under control of the steroid hormones of the ovary, it is logical to consider use of these same steroids to correct excessive uterine bleeding due to disorders of cyclic ovarian-endometrial function. The literature in the past decade has reflected extensive study of endocrine therapy for functional uterine bleeding; and judicious use of gonadal steroids has finally gained warranted prestige over radiation and radical surgical procedures in the treatment of this one of the most common of the endocrinopathies.

Thorough and complete physical examination is necessary in order to exclude all possible organic and systemic causes of uterine bleeding, *e.g.*, malignant neoplasms, cervical polyps, complications of conception, blood dyscrasias, infection, and metabolic factors such as hypothyroidism. Functional uterine bleeding has been defined as abnormal and excessive bleeding which occurs because of physiologic disturbances and not because of pathologic processes (3, 4). Although hormonal dysfunction is the chief cause, nutritional factors, vitamin deficiencies, and nervous and psychogenic factors play important contributory roles. Pelvic inflammatory disease, endometriosis, endometrial polyps, and frequently, fibromyomas of

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the uterus may now be included among the causes of functional uterine bleeding, for excessive bleeding associated with these conditions responds to therapy in a manner similar to that of purely functional disturbances (3). It may be postulated that these latter conditions are responsible for ovarian or end-organ (endometrial) dysfunction, and the actual mechanism of bleeding may be the same as in the purely functional form.

Mechanism of uterine bleeding

It is believed that menstruation is the result of withdrawal or decrease of a stimulus necessary to maintain the condition of the endometrium at the end of the cycle (5); hence, bleeding terminating an ovulatory cycle is due to withdrawal or decrease in estrogen and progesterone. Hormonal withdrawal, then, permits changes in the endometrium and its blood vessels which result in tissue fragmentation, bleeding, and shedding of the endometrium (6, 7).

Failure of ovulation and persistence of the unruptured follicle results in prolonged estrogenic stimulation of the endometrium in absence of progesterone (8–12). The amount of bleeding cannot be correlated with the degree of endometrial hyperplasia, probably because of individual differences in endometrial response to estrogen (13).

Tension within the persistent follicle eventually results in atrophy of the granulosa cells with decrease in estrogen production and associated estrogen-withdrawal bleeding. Bleeding under these conditions, from an hyperplastic proliferative endometrium, in absence of menstrual toxin is not only unpredictable as to onset and duration, but disintegration of the endometrium is focal and prolonged rather than diffuse and more or less "explosive," as is the case in normal postovulatory shedding (14). The ovarian dysfunction may be primary, or secondary to inadequate pituitary stimulus due to an abnormal constitutional or hypothalamic state.

Rationale of therapy

A sufficient number of clinical studies has appeared in the literature to warrant acceptance of the fact that properly administered estrogenic hormone will stop acute functional uterine bleeding. Many, however, are of the opinion that use of estrogen in the therapy of menometrorrhagia is not physiologic, since many cases of functional uterine bleeding are the result of endometrial hyperplasia believed to be due to prolonged stimulation by this same steroid (15–17). On the other hand, there are many investigators who advocate the use of estrogen in the control of uterine hemorrhage (18–21). Novak (22) has pointed out that there is no parallelism between the amount of bleeding and the degree of endometrial histologic response. Kurzrok (23), in agreement with many others, has pointed

out that functional bleeding may occur from any type of endometrium, namely, proliferative, cystic glandular hyperplasia, progestational, acyclic, and even atrophic endometrium. Furthermore, any of these phases of endometrial response may also be associated with amenorrhea.

The hemostatic effect of estrogen administered orally or by injection has been attributed to an inhibitory effect on the ovary caused in turn by inhibition of the pituitary gonadotropic hormone. It has also been suggested that administration of estrogen controls excessive uterine bleeding by raising the estrogen level in the circulating blood above the so-called "critical level" (20). Large doses of estrogen, either orally or by injection, have frequently been reported to stop uterine hemorrhage. Six to ten days after cessation of therapy, however, uterine bleeding has often recurred, and has frequently been as profuse as the initial hemorrhage.

In recent years convincing evidence of satisfactory results employing either estrogen, progesterone (24), or testosterone, or these steroids in combination, have appeared in the literature. Although we have found the combinations of testosterone-progesterone, and estrogen-progesterone-testosterone quite satisfactory in "the arrest of bleeding according to plan" (3)(25), the subsequent period of withdrawal bleeding may be undesirable in many cases; and since potent preparations of estrogen for intravenous administration have been made available, we believe that clinical evaluation of this addition to our armamentarium has been warranted. Our results confirm and extend those of Zondek (26) and Abarbanel (27).

ADMINISTRATION OF ESTROGEN INTRAVENOUSLY FOR CONTROL OF ACUTE UTERINE BLEEDING

Clinical evaluation. Sixty episodes of uterine bleeding in 56 patients, ranging from 15 to 56 years of age, were treated by intravenous administration of estrogen (Table 1). A careful medical history was obtained, and a complete physical examination was performed on each patient. Vaginal and cervical smears were obtained for cytologic study according to the Papanicolaou technique, and these were repeated after initial control of bleeding. Endometrial biopsy specimens were obtained by aspiration curettage. Capillary fragility tests and complete blood counts were made routinely.

The usual dosage employed was 5 cc. of an aqueous solution containing 20 mg.[1] of conjugated natural estrogens (equine) (Premarin(R)) intravenously.

During the early part of the study this dosage was repeated one to three times at daily intervals; but as observation of results continued, it soon became apparent that administration of 20 mg. intravenously every

[1] Expressed as sodium estrone sulfate.

six to twelve hours for two to four doses—or until cessation of bleeding—proved more satisfactory. During the interval that followed cessation of bleeding, continued diagnostic efforts, when necessary, and steps toward correction of the underlying cause of the condition were pursued. For prevention of estrogen-withdrawal bleeding after initial control of menorrhagia, Premarin(R) 1.25 mg. (or its equivalent) was given orally four times daily for five days, then decreased to three times daily for five days, then twice daily for five days, and finally, 1.25 mg. daily for five days. With this

TABLE 1. RESPONSE TO THERAPY

Diagnosis	No. of courses of therapy	Response to intravenous estrogen therapy		
		Satisfactory	Temporary	Failure
1. Functional uterine bleeding	57	48	5	4
2. Adenocarcinoma of body of uterus	1	—	1	—
3. Ectopic pregnancy (tubal)	2	—	2	—

gradually diminishing dosage schedule, recurrence of bleeding was infrequent; and when it occurred, dosage was increased to the previous higher level with satisfactory arrest of bleeding. Patients approaching the menopause were then given 0.625 mg. of Premarin(R) orally for an additional week and rarely required further therapy. In younger patients, however, to prevent recurrence of menorrhagia due to ovulatory failure and endometrial hyperplasia, this schedule was followed by a course of progesterone therapy[2] to induce bleeding with shedding of the endometrium. Cyclic progesterone therapy (frequently after a course of oral estrogens) was then administered each month until spontaneous ovulation was resumed. Return of ovulatory function was determined by interpretation of biphasic basal temperature records, and confirmed upon observing a progestational endometrium in a specimen obtained by aspiration curettage on the first day of subsequent spontaneous menstruation.

Until an optimal dosage of the intravenous estrogen and an adequate follow-up schedule were determined by observation of results in the early cases of this series, all patients who failed to respond to an injection (20 mg. of Premarin(R)) daily for a period of two or three days were successfully treated with the estrogen-progesterone-testosterone combination (3).

[2] Progesterone by ingestion, 30 mg. three times daily for five days (28); or anhydrohydroxyprogesterone by ingestion, 10 mg. three times daily for five days; or progesterone (prepared for buccal administration), 10 mg. three times daily for five days.

Later in the study, however, no failures were encountered when adequate amounts (20 mg.) of intravenous estrogen were administered at 6-hour to 12-hour intervals; and no patient required in excess of four injections of 20 mg. each. There were no untoward effects of therapy with intravenous administration of conjugated estrogens.

Discussion of results. In this study the results of therapy consistently improved as our experience with administration of estrogen intravenously increased. Rapid decrease in the amount of uterine bleeding was noted within three to twelve hours after initial intravenous injection of 20 mg. of conjugated estrogens (equine) in 48 out of 57, or 84.2 per cent of courses of therapy in patients with functional uterine bleeding. Four failures occurred early in the study, and in 3 of these (Table 2, Cases 4, 11 and 24) the patient received only one I.U. injection of estrogens. The fourth patient received only one additional injection of 20 mg. twenty-four hours after the initial dose. Since little or no decrease in the amount of bleeding was noted in these patients they were recorded as failures; but 3 of them responded satisfactorily to the estrogen-progesterone-testosterone combination. The other (Table 2, Case 17), a case of menorrhagia occurring several weeks after a spontaneous abortion, showed increased bleeding after therapy. In 5 other instances (Table 2, Cases 13, 19, 20, 25 and 26) there was a temporary decrease in bleeding but the overall result was unsatisfactory. In 2 cases of ectopic pregnancy (Table 2, Cases 21 and 29) and 1 of adenocarcinoma of the endometrium (Table 2, Case 45) there also was temporary improvement but spotting or bleeding continued.

Later patients, in whom no apparent response occurred in six hours, were given repeated injections of 20 mg. of Premarin(R) every six to twelve hours, and no failures were observed when this regimen was carried out. No difficulties were encountered in follow-up therapy of diminishing dosage of estrogen orally, except an occasional complaint of nausea which was in no case intolerable and tended to disappear after a few days of continued therapy. This was minimized by administration of the oral estrogen immediately after meals and at bedtime.

Functional uterine bleeding

Results of therapy. In the treatment of acute menorrhagia associated with hormonal imbalance there was cessation or satisfactory reduction in the amount of bleeding within three to twelve hours after intravenous administration of 20 mg. of Premarin(R) at intervals of six to twenty-four hours, in 48 out of 57 courses of therapy. Endometrial biopsies in this group revealed primarily cystic glandular hyperplasia, or imperfect progestational changes, although one biopsy specimen obtained by aspiration curettage showed an endometrial polyp (Case 6). Other conditions asso-

TABLE 2. UTERINE BLEEDING

No.	Age	Duration of bleeding	Endometrium*	Intravenous estrogen (mg.)	Results	Remarks
1. M.C.	24	11 days	E+	20	Stopped within 12 hrs.	Cyclic therapy 2 mos. Ovulation resumed
2. M.W.	26	17 days	E+	20	Stopped within 10 hrs.	
3. E.R.	30	60 days		20	Stopped within 48 hrs.	Fibromyomata
4. D.H.	28	22 days		20	No change	PID, inadequate therapy
				(24 hrs.) 20	No change	
5. M.A.	31	Irreg. heavy, 6 mos.		20	Stopped in 6 hrs.	Fibromyomata; Hgb 5 Gm.
6. M.S.	25	11 days	E+ Endometrial polyp	20	Stopped in 6 hrs.	Cyclic therapy 3 mos.; regular
7. P.R.	36	16 days	E (Following estrogen therapy)	20	Stopped in 36 hrs. (for 6 hrs.)	Cyclic therapy
				(48 hrs.) 20	Stopped in 8 hrs.	
8. G.B.	25	21 days	E+	20	Stopped in 3 hrs.	
9. M.S.	29	90 days	E+	20	Stopped in 72 hrs. (for 23 days)	No Rx
10. M.S.	29	10 days	E+	20	Stopped in 4 hrs.	Cyclic therapy
11. M.L.	28	19 days		20	No change	Inadeq. Rx; neurofibromata
12. M.B.	31	9 mos.	P−	20	Stopped in 72 hrs.	Cyclic therapy
13. O.	39	21 days		20	Stopped in 8 hrs. (for 1 day)	Hypermenorrhea for years; inadeq. Rx
				(36 hrs.) 40	Stopped in 8 hrs. (for 1 day)	
14. C.J.	30	14 mos.	P−	20	Stopped in 6 hrs.	Oral progest.; normal cycle
15. M.S.	20	11 days	E	20	Stopped in 12 hrs.	Cyclic therapy
16. A.C.	16	12 days	E	20	Stopped in 3–4 hrs.	Desc. estrogen (cretin)
17. P.S.	23	8 days	P−	20	Increased bleeding	Abortion sublinvolution
18. H.	32	8 days		20	Stopped in 8 hrs.	Cyclic Rx
19. C.C.	34	19 days		20	Decreased in 12 hrs.	Inadeq. Rx; hypermenorrhea Normal; androgen Rx
20. K.I.	32	4 days	E+	20	Decreased in 6 hrs.	Endometriosis; inadeq. Rx
				(6 hrs.) 20	Stopped for 3 hrs.	Responded to EPT
21. M.W.	35	12 days	E+	20	Stopped in 8 hrs. (for 24 hrs.) Stopped in 6 hrs.	Inadequate therapy Cyclic therapy
22. L.L.	21	14 days	E	20	Stopped in 6 hrs.	Oral estrogens
23. L.L.	21	8 days	E+	20	Stopped in 24 hrs.	Cyclic therapy Primary hypermenorrhea
24. L.B.	30	52 days	E+	20	No change	Inadequate therapy Responded to EPT
25. M.G.	24	49 days		20	Decreased in 6 hrs.	Inadequate therapy Responded to EPT
26. J.L.	26	30 days		20	Decreased in 5 hrs.	Inadequate therapy Responded to EPT
27. F.S.	23	20 days		20	Decreased in 12 hrs.	
				(24 hrs.) 20	Stopped in 36 hrs. (for 6 days)	Ectopic pregnancy
28. L.B.	24	14 days		20	Stopped in 24 hrs.	
29. O.M.	23	12 days		20	Slowed in 8 hrs.	
				(24 hrs.) 20	Spotting only	Ectopic pregnancy
30. R.M.	56	5 days	E+	20	Stopped in 12 hrs.	Menopause; estrogen Rx
31. J.P.	40	14 days	P−	20	Stopped in 24 hrs.	Fibroids—excessive oral prog. w/d bleeding
32. M.S.	40	60 days		20	Spotting only after 8 hrs.	Fibromyomata
				(18 hrs.) 20	Stopped in 6 hrs.	

* Key: E Proliferative
E+ Cystic glandular hyperplasia
E− Atrophic endometrium
P Progestational
P− Mixed, or imperfectly progestational

TABLE 2—(*continued*)

No.	Age	Duration of bleeding	Endometrium*	Intravenous estrogen (mg.)	Results	Remarks
33. J.W.	20	8 days		20	Stopped in 8 hrs.	
34. C.W.	50	5 mos.	E+	20	Slight, 4 hrs. later	
				(4 hrs.) 20	Decreased after 12 hrs.	
				(24 hrs.) 20	Spotting only	
				(26 hrs.) 20	Stopped in 2 hrs.	Fibromyomata
35. O.S.	32	60 days		20	Stopped in 12 hrs.	Cyclic therapy
36. E.C.	24	10 days		20	Stopped in 12 hrs.	PID
37. R.C.	28	18 days		20	Stopped in 36 hrs.	PID
38. D.B.	21	30 days	E+	20	Stopped in 36 hrs.	
39. R.H.	25	21 days		20	Stopped in 36 hrs.	Cyclic therapy
40. I.J.	21	14 days		20	Decreased after 24 hrs.	Did not take oral estrogen
				(24 hrs.) 20	Stopped in 8 hrs. (for 10 days)	as prescribed
41. I.J.	21	5 days		20	Stopped in 6 hrs. (for 10 days)	PID
42. I.J.	21	4 days		20	Stopped in 48 hrs.	PID
43. T.A.	27	16 days	E+	20	Decreased after 24 hrs.	
				(24 hrs.) 20	Stopped in 4 hrs.	
				(24 hrs.) 20	No bleeding	Followed by progesterone;
				(24 hrs.) 20	No bleeding	then normal menses
44. E.A.	28	33 days		20	Stopped in 24 hrs.	
				(24 hrs.) 20	No bleeding	
45. L.H.	49	67 days	Ca	6	Decreased in 24 hrs.	
				(24 hrs.) 8	Spotting	Adenocarc. body of uterus;
				(24 hrs.) 8	Stopped for 24 hrs.	hysterectomy
46. H.R.	27	71 days	E+	20	Decreased in 24 hrs.	
				(24 hrs.) 20	Stopped	
				(24 hrs.) 20	No bleeding	
47. J.W.	42	Cyclic	P	20	Spotting after 12 hrs.	
		menorrhagia		(12 hrs.) 20	Stopped	Cyclic therapy
48. A.R.	39	14 days	P	20	Slowed in 5 hrs.	
				(6 hrs.) 20	No bleeding	Cyclic therapy
49. P.M.	37	12 days		20	Stopped within 12 hrs.	
				(12 hrs.) 20	No bleeding	Cyclic therapy
50. J.D.	32	14 days		20	Stopped in 4 hrs.	
				(4 hrs.) 20	No bleeding	Cyclic therapy
51. A.C.	33	6 mos.		20	Decreased in 5 hrs.	
		poly-		(5 hrs.) 20	Stopped in 7 hrs.	Cyclic therapy
		menorrhea				
52. E.A.	26	10 days		20		
				(8 hrs.) 20		
				(12 hrs.) 20	Stopped in 12 hrs.	Cyclic therapy
53. K.I.	32	11 days	E+	20		Bleeding after estrogen
				(6 hrs.) 20		Rx for endometriosis
				(6 hrs.) 20	Decreased in 6 hrs.	
				(12 hrs.) 20	Stopped 12 hrs. after initial dose	
54. R.D.	28	21 days		40	Spotting at 6 hrs.	
				(6 hrs.) 20	Stopped in 12 hrs.	
				(14 hrs.) 20	No further bleeding	Cyclic therapy
55. T.W.	39	12 mos.		20	Spotting only, in 16 hrs.	
		menomet-		(16 hrs.) 20	Stopped in 2 hrs.	
		rorrhagia		(22 hrs.) 20	No bleeding	Cyclic therapy
56. J.T.	25	9 days		20	Stopped in 12 hrs.	Cyclic therapy
57. L.B.	34	14 days	E+	20	Stopped within 12 hrs.	Cyclic therapy
58. W.B.	31	18 days	E−	20	Spotting only, in 8 hrs.	
		(irreg.		(17 hrs.) 20	Spotting only	
		8 mos.)		(24 hrs.) 20	Stopped	
59. J.A.	28	14 days	P	20	Spotting only, in 3 hrs.	
		(spotting		(3 hrs.) 20	No bleeding in 5 hrs.	
		5 mos.)		(12 hrs.) 20	No bleeding	
				(6 hrs.) 20	No bleeding	Cyclic therapy
60. S.R.	15	2 days		20	Spotting only, in 3 hrs.	
				(3 hrs.) 20	Stopped within 2 hrs.	

ciated with menorrhagia which were included in this group were fibromyomas, pelvic inflammatory disease, Von Recklinghausen's disease (generalized neurofibromatosis), menopausal menorrhagia, estrogen-withdrawal bleeding following menopausal therapy and following estrogen therapy for endometriosis, and excessive progesterone-withdrawal bleeding in the presence of fibromyomas of the uterus. The response to therapy in these conditions was, in general, uniform and satisfactory. We felt that the 3 patients in whom there was no significant decrease in bleeding received inadequate therapy, since only 1 of these received a second injection, and this was not given until twenty-four hours later.

Response to follow-up therapy in these cases was uniformly satisfactory except for 1 instance (out of 48) (Table 2, Case 40). This patient did not take the oral estrogen as prescribed, and bleeding recurred ten days after initial therapy.

It is of interest to note that the patient with the endometrial polyp associated with cystic glandular hyperplasia (Table 2, Case 6) had been bleeding excessively for eleven days, and stopped completely within six hours after a single intravenous injection of 20 mg. of Premarin(R). This patient was started immediately on the usual course of Premarin(R), 1.25 mg. orally four times daily for twenty days, followed by anhydrohydroxyprogesterone, 10 mg. three times daily for five days. Two days after completion of this therapy a normal 5-day menstrual period began. The anhydrohydroxyprogesterone therapy was repeated from the twentieth to the twenty-fifth day of each of the next two cycles, with the same response. In the third month, however, the basal temperature record revealed a biphasic graph, therapy was discontinued, and a regular ovulatory cycle was resumed.

One 31-year-old patient, with multiple large fibromyomas of the uterus (Table 2, Case 5) gave a history of irregular heavy bleeding for six months. On examination there was massive uterine bleeding. She was in shock, and blood studies revealed only 5 grams of hemoglobin. These circumstances obviously made the patient a poor surgical risk. The bleeding completely stopped within six hours after a single injection of 20 mg. of conjugated estrogens intravenously, during which time the patient received by transfusion 3 pints of whole blood. Before this trial of hormonal therapy, transfusions proved of little avail, because the benefits derived from the infused blood were gradually undone by the persistent loss of blood by way of uterine bleeding. Hysterectomy and convalescence were uneventful.

Bleeding due to organic causes

Ectopic pregnancy—Failure of typical response to therapy; value in early diagnosis. To emphasize the importance of repeated examinations and careful evaluation of symptoms of each patient during the course of ther-

apy, and to point out that failure of a typical response to therapy may be a valuable diagnostic aid, 2 patients who later were found to have tubal pregnancies are included in this series. One (Table 1, Case 27), a 23-year-old married nullipara, gave a history of laparotomy for tubal pregnancy five months prior to her present illness. After two normal menstrual periods, the third continued for twenty days. There had been no pain, and pelvic examination showed nothing abnormal except for bleeding from the cervix. The pregnancy tests at that time gave negative results. Uterine bleeding slowed to slight spotting within twelve hours after initial injection of 20 mg. of Premarin(R) intravenously, and stopped completely within twenty-four hours after the second injection, which was given the next day. Oral administration of Premarin(R), 1.25 mg. four times daily, was started after the initial injection. Heavy bleeding recurred six days later on discontinuation of oral estrogen because of severe nausea. However, at this time, pelvic examination showed questionable enlargement and slight tenderness of one Fallopian tube, a condition which had not been noted on previous examination. The pregnancy tests (frog and rat tests) at this time gave positive results, and laparotomy revealed an ectopic pregnancy in the remaining tube.

The other patient (Table 2, Case 29), a 23-year-old nulligravida, gave a history of menstrual irregularity at intervals varying from three to six weeks. The previous menstrual period was normal six weeks prior to onset of her current complaint of heavy bleeding with large clots for twelve days. Pelvic examination revealed a moderately tender adnexal mass which was interpreted to be a slightly enlarged ovary, although the possibility of early ectopic pregnancy was not overlooked. The patient was examined daily thereafter. Intravenous administration of 20 mg. of Premarin(R) was followed by rapid cessation of excessive bleeding, and at the time of a second injection eight hours later, only slight spotting was noted on examination. This spotting continued, however, in spite of the usual oral estrogen therapy throughout the succeeding forty-eight hours, after which time the adnexal mass was found to be slightly larger and considerably more tender. The results of the pregnancy tests were reported to be positive, and laparotomy confirmed the diagnosis of early ectopic gestation in the fimbriated portion of the Fallopian tube, adherent to the ovary.

These 2 cases are presented to stress two important factors in therapy: 1) the necessity of persistent effort in order to determine the cause of menorrhagia, and 2) even though response to intravenous therapy for the acute hemorrhage may be satisfactory, failure of the typical satisfactory response to follow-up therapy demands careful re-evaluation and further study.

Menorrhagia associated with subinvolution of the uterus following spon-

taneous abortion. A 23-year-old patient gave a history of continued spotting for several weeks after a spontaneous early abortion, and then heavy bleeding for eight days. An endometrial biopsy specimen obtained by aspiration curettage revealed an imperfect progestational endometrium. Uterine bleeding increased in amount following administration of 20 mg. of Premarin(R) intravenously. The bleeding lasted for five days and stopped. Follow-up evaluation suggested that this episode may have been an ovulatory menstrual period, although moderate subinvolution of the uterus cannot be ignored as a probable factor in the failure of response to therapy.

Adenocarcinoma of the body of the uterus was ascertained by routine endometrial biopsy (aspiration curettage)[3] performed during the initial examination of a 49-year-old patient who gave a history of slight to moderate bleeding for approximately two months (Table 2, Case 45). Although for no particular reason this patient was given less than the usual dosage of intravenous estrogens, it is interesting to note that in spite of the etiology in this case, there was temporary arrest of bleeding following therapy. The amount of bleeding was considerably diminished within twenty-four hours after an initial intravenous injection of 6 mg. of Premarin(R), and was reduced to slight spotting within twenty-four hours after the second injection (8 mg.) the next day. The last injection (8 mg.) was given the following day, and there was no bleeding for twenty-four hours. By this time the diagnosis was evident from pathologic study of the endometrial biopsy specimen, and surgical therapy was instituted.

SUMMARY AND CONCLUSIONS

1. Sixty episodes of acute uterine bleeding were treated by the intravenous administration of an aqueous solution containing Premarin(R) (Conjugated Natural Estrogens—equine). The usual dosage employed was 5 cc. (containing 20 mg. of estrogenic substances expressed as sodium estrone sulfate) every six to twelve hours for a total of three or four doses.

2. Persistent efforts to determine the cause of menorrhagia included complete medical histories and physical examinations, complete blood counts, capillary fragility tests, endometrial biopsies in many cases (as-

[3] Aspiration or suction curettage (an office procedure) is performed in lieu of a regular curettage (a hospital procedure). In our hands, suction curettage has proved adequate. Whenever a patient fails to respond to our hormonal regimen, then further steps are taken. In such instances a dilatation and curettage should be undertaken to rule out malignancy. Since submitting this paper, 2 more cases of endometrial carcinoma have been diagnosed through suction curettage. Incidentally, both of these patients failed to respond adequately to therapy and, therefore, immediate steps were taken for proper management.

piration curettage), pregnancy tests, and repeated daily pelvic examinations with particular attention to evaluation of response to therapy.

3. The response to intravenous estrogen therapy was observed in menorrhagia associated with various conditions, including functional uterine bleeding associated with cystic glandular hyperplasia of the endometrium, acyclic and imperfect progestational endometrium, uterine fibromyomas, estrogen-withdrawal bleeding, progesterone-withdrawal bleeding (excessive, in presence of fibromyomas), endometriosis, endometrial polyp, Von Recklinghausen's disease, menopausal menorrhagia, pelvic inflammatory disease; also from organic causes, including menorrhagia associated with post-abortal subinvolution of the uterus, ectopic pregnancy, and adenocarcinoma of the body of the uterus.

4. Satisfactory arrest of bleeding was noted in 48 of the 57 courses of therapy for functional uterine bleeding. In 5 others, the results were temporary and the overall result was inadequate. A warning is made that great care should be exercised in the management of patients with uterine bleeding since apparently good, though temporary, results may be obtained in endometrial carcinoma or ectopic pregnancy.

5. After initial arrest of bleeding by administration of estrogens intravenously, a regimen of oral estrogen therapy in diminishing doses followed by a course of cyclic progestin therapy was satisfactory in re-establishing the menstrual cycle.

6. No untoward effects of therapy with intravenous administration of conjugated estrogens were encountered, except for some nausea.

7. From the results of this study it may be concluded that estrogens prepared for intravenous administration may be successfully employed in the arrest of functional uterine bleeding. This form of therapy is particularly valuable for certain cases in which withdrawal bleeding is undesirable.

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THE EFFECT OF INTRAVENOUS TESTOSTERONE ON NITROGEN AND ELECTROLYTE METABOLISM*

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INTRODUCTION

THE anabolic effects of testosterone have been studied in some detail in both man and experimental animals since the original demonstration by Kochakian (1, 2, 3) that testosterone caused nitrogen retention in castrated dogs. This anabolic effect was subsequently observed in both eunuchoid and normal men by Kenyon and his coworkers (4, 5). The latter investigators also observed a retention of potassium, sodium, inorganic phosphorus, sulfate, and chloride during testosterone therapy.

Because of its extremely poor solubility in aqueous solutions, testosterone was administered intramuscularly in oil in these studies. Bischoff's demonstration (6) of the high solubility of testosterone in serum albumin suggested the possibility of administering large amounts of testosterone dissolved in human serum albumin solution in a single intravenous dose. The present investigation was undertaken to determine whether testosterone in this form would have any effect on nitrogen and electrolyte metabolism in normal men.

METHODS

Four normal men were used in these studies. A constant diet of known nitrogen and electrolyte content was given daily throughout each of the

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respective experiments. When nitrogen equilibrium was established, a single intravenous dose of testosterone in 25 per cent human serum albumin was given.

Throughout the experimental period urine was collected every twenty-four hours and analyzed for various constituents. Nitrogen, creatine and creatinine were determined in all cases, phosphorus in 2, and sodium and potassium in 1. In addition, all stools were collected and their nitrogen and phosphorus content determined. An aliquot of the diet was also analyzed for its nitrogen and electrolyte content.

Nitrogen determinations were made by the macro Kjeldahl technique. The Fiske and SubbaRow method was used for phosphorus determinations (7). Creatine and creatinine were measured by the Jaffé reaction (8). Sodium and potassium were estimated by means of a Perkin-Elmer flame photometer. The Callow modification of the Zimmermann reaction (9) was used to measure 17-ketosteroids.

The testosterone-albumin solutions were assayed for testosterone by extracting aliquots with ethyl ether. The ether extracts were dissolved in absolute ethanol and their absorption in the ultraviolet range measured. Steroids having an α, β-unsaturated ketone structure, such as testosterone, absorb maximally at 240 mμ. The concentration of testosterone in the solutions was determined by the absorption at this wave-length. Samples of blood were analyzed for the hormone by the technique of West (10).

RESULTS

A normal 24-year-old male (Case 1) received 162 mg. of testosterone intravenously after nitrogen and phosphorus equilibria were established. The effects on nitrogen, phosphorus, and water balances are indicated in Figure 1. In the method of graphing used for nitrogen and phosphorus balances throughout this paper, the dietary intake is indicated below the zero line and the excretion is plotted upward from the dietary value. Thus excretion values above the zero line indicate a negative balance and those below, a positive balance. Inasmuch as the absolute water intake and output were not determined, the fluid intake minus the urine volume has been plotted. Assuming that other routes of water excretion and intake were constant, these figures will vary as the water balance.

After the injection of the hormone there was a significant increase in the water retained, beginning on the second day and continuing thereafter for four days. The excess water did not appear to be lost during the remainder of the observation period.

As indicated by the spaces below the zero line, there was a positive nitrogen and phosphorus balance for ten days after testosterone administration. During this period there was a retention of 16.2 Gm. of nitrogen and 1.06

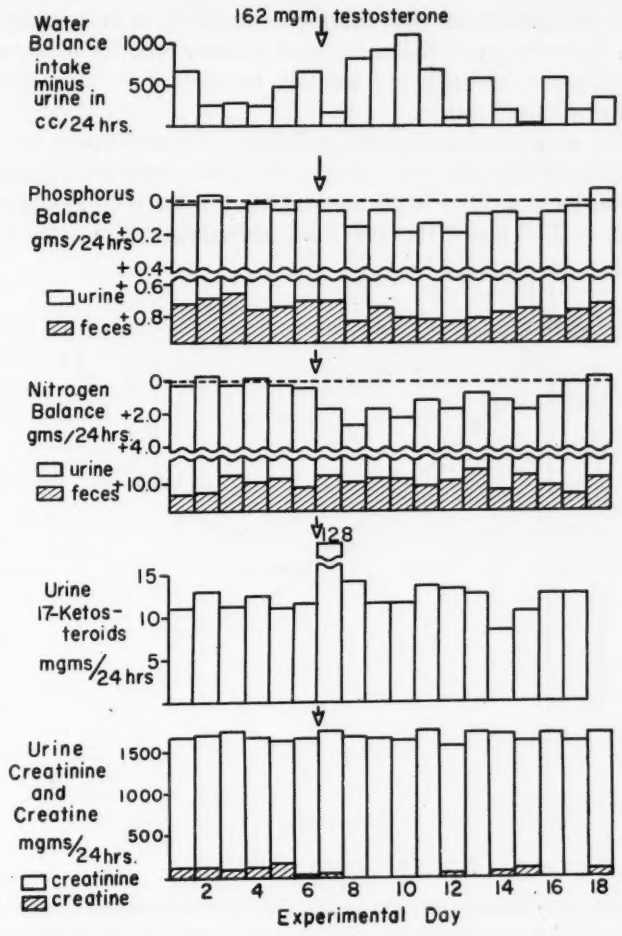

FIG. 1. *Case 1.* Effect of intravenous injection of testosterone dissolved in 25 per cent human albumin solution on water, phosphorus and nitrogen balances and excretion of 17-ketosteroids, creatine and creatinine.

Gm. of phosphorus. The maximum 24-hour retention of nitrogen occurred on the second day and amounted to 2.8 Gm.; that for phosphorus occurred on the fourth day and amounted to 0.19 Gm.

Most of the testosterone was excreted as excess 17-ketosteroids within twenty-four hours after administration. The 17-ketosteroid excretion again rose slightly higher than that of the control period on the fifth to seventh days of the postinjection period.

Figure 1 also illustrates that there was no effect on creatinine excretion in Case 1. However, the small amount of urinary creatine observed during the control period disappeared entirely for four days after intravenous testosterone administration.

As shown in Figure 2, the concentration of testosterone in the blood fell rapidly after the injection. After a 90-minute interval it was no longer measurable. Only a very small amount of α, β-unsaturated ketosteroids, equivalent to 2.06 mg. of testosterone, appeared in the urine during the

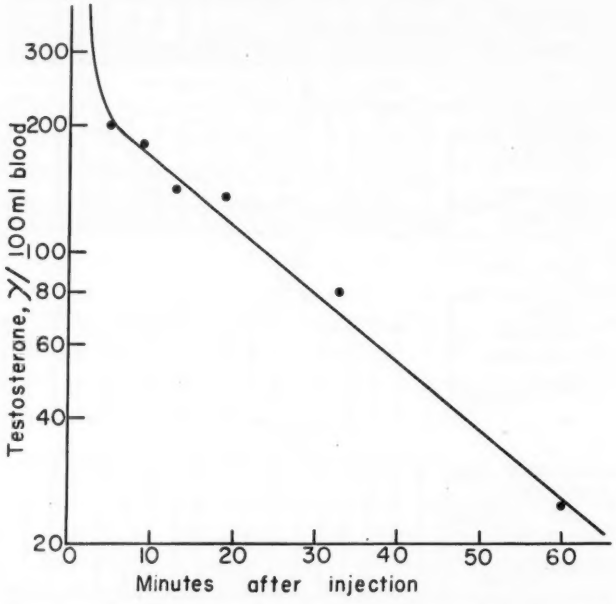

FIG. 2. Levels of testosterone in the blood (Case 1) after the intravenous injection of 162 mg. of testosterone in 25 per cent serum albumin solution.

first hour and none appeared thereafter. Direct excretion, therefore, could not account for the disappearance of the injected hormone.

Hourly urine collections were made for six hours after administration of testosterone. Each sample was analyzed for nitrogen, creatinine, creatine, phosphorus and 17-ketosteroids to determine the relation of the metabolic degradation of testosterone to the changes in tissue metabolism. Large amounts of 17-ketosteroids appeared in the urine during the first two hours and then fell off rapidly. The results are illustrated in Figure 3. No alteration was observable in the excretion of nitrogen or creatinine during the six-hour period. Urinary creatine, however, began to fall within an

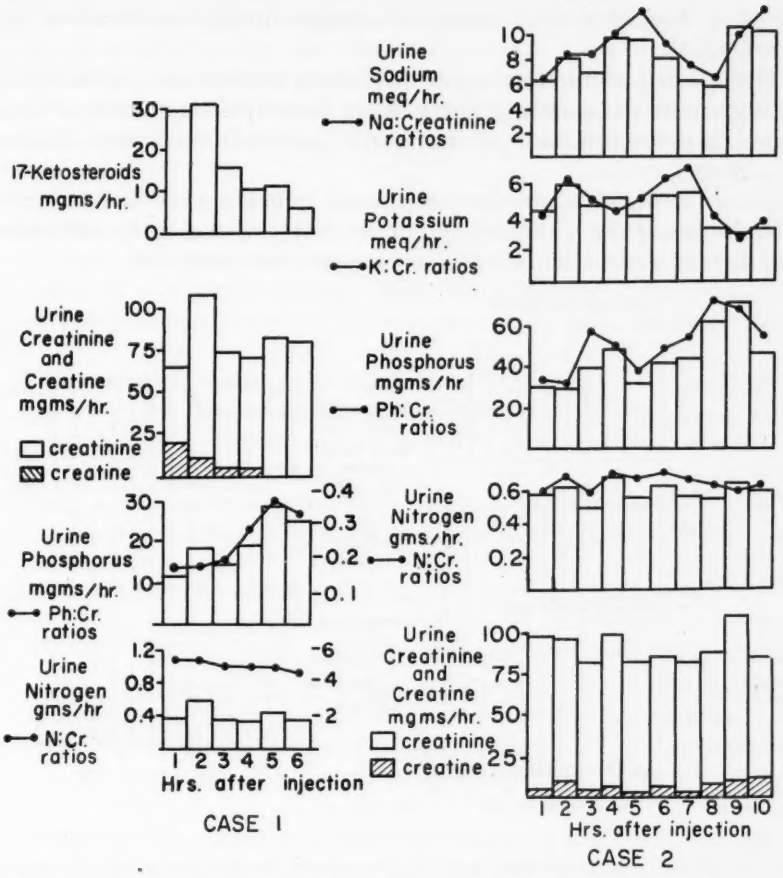

FIG. 3. Hourly excretion of various metabolites after the intravenous injection of testosterone dissolved in 25 per cent serum albumin solution.

hour and had disappeared from the urine by the fifth hour. Phosphorus began to rise by the fourth hour, both in absolute amounts and in relation to creatinine excretion. It would seem, then, that these changes antedated the significant storage of nitrogen.

In the second subject, after nitrogen balance was established, a solution containing 192 mg. of testosterone dissolved in a solution of human serum albumin was administered over a period of fifteen minutes. The various components were measured in the urine for eight days thereafter.

As observed in the first subject, there was an immediate positive nitrogen balance. This reached a maximum on the second day and continued

for seven days. The total storage of nitrogen during this time was 14.1 Gm. (Fig. 4).

The injected testosterone again was largely excreted as 17-ketosteroids during the first twenty-four hours. As in Case 1, there seemed to be an approach to the control levels during the third and fourth days, followed by a secondary rise.

As seen in Figure 4, creatine disappeared from the urine four days after the injection of the hormone and did not reappear during the subsequent four days of observation. Creatinine excretion was unaffected.

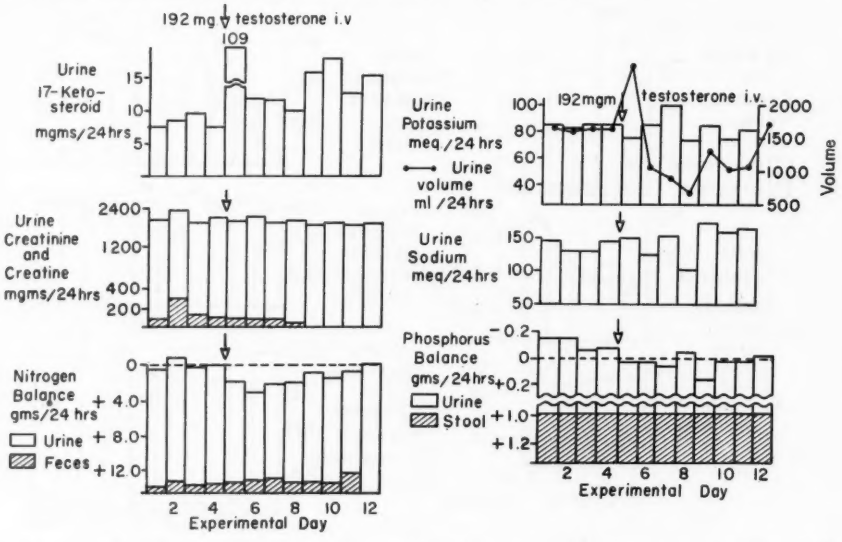

FIG. 4. *Case 2.* Effect of intravenous injection of testosterone on nitrogen and phosphorus balances, and excretion of 17-ketosteroids, creatine, creatinine, sodium and potassium.

The excretion of water and electrolytes is also illustrated in Figure 4. In this instance both food and water intake were maintained at a constant level. The drop in urine volume from the second to seventh day after testosterone again indicates a prolonged retention of water after the single injection of testosterone.

Phosphorus retention was also immediate and prolonged. During the control period phosphorus was apparently in continuous negative balance but the patient began to store it immediately after the injection of testosterone and it remained predominantly in positive balance until the eighth day. Excretion of sodium and potassium seemed to fluctuate more after treatment than before, and no significant overall change could be established.

The excretion of the various substances was followed hourly for ten hours after the injection. The results are shown in Figure 3. The hourly output of sodium varied considerably but seemed to show a tendency to rise over the periods measured, based either on absolute amounts or on the ratio to creatinine excretion. The opposite trend was observable with potassium.

The significant rise in phosphorus excretion observed in Case 1 was again noticed in these hourly values, maximum excretion being reached at the ninth hour. Nitrogen and creatinine output again showed no significant change over this period of time. Unlike the first subject, the second continued to show small amounts of creatine in each of the ten hourly collections.

Since prolonged nitrogen, phosphorus and water retention had followed the single injection of 160–200 mg. of testosterone dissolved in a solution of serum albumin, a study was made of the effect of smaller amounts of the hormone administered intravenously. In the third case an injection of 20.6 mg. was given to a normal young man. There was no significant storage of nitrogen during the experimental period. Apparently a single dose of this size is ineffective.

In another normal young male (Case 4), 101 mg. of the steroid was injected. There was a positive nitrogen balance of questionable significance for the first two days after administration, followed by a slight negative balance for four days. Certainly the effect was not directly proportional to the size of dose. It would appear that it is the high concentration of testosterone in the blood, achieved by the larger doses, which is important in producing the prolonged metabolic changes noted in the first 2 subjects.

DISCUSSION

The results of this investigation indicate that intravenous injection of testosterone, like intramuscular injections, causes an increased formation of cellular tissue, since nitrogen, phosphorus and water are stored. Unlike the effect of intramuscular injections however, the reduced excretion of these components is immediate and prolonged after only one dose. When testosterone in doses of 25 mg. per day is given intramuscularly to normal young men there is a gradual fall in the excretion of nitrogen which after four to eight days reaches a positive balance similar in magnitude to that seen on the second day in these experiments (5). If intramuscular injections are continued until this level is reached, the return to equilibrium on cessation of treatment resembles the nitrogen balance curves in Cases 1 and 2 after the second day. In other words, the intravenous injection achieves in one day the effect observed after four to eight days of daily intramuscular injections, and apparently with about the same total dose.

The storage of nitrogen does not seem to have been related to the conversion of testosterone to 17-ketosteroids. The greatest part of the injected hormone was accounted for by the 17-ketosteroids which were excreted during the first twenty-four hours, over half of which appeared during the first six hours. Yet during the six-hour period there was no significant change in nitrogen excretion. The only other consistent change in the first six hours was an increase in the output of phosphorus, a change in the opposite direction from that which occurred later during the storage of nitrogen.

Another fact to be considered in this connection is the failure of the lower doses to effect a proportionate change in nitrogen balance. With these doses the blood level was increased only briefly; the hormone was rapidly cleared from the blood by conversion to 17-ketosteroids. It seems, then, that the formation of tissue, which is suggested by the positive nitrogen balance, is dependent on the fixation of the hormone in the tissues when the higher doses are given intravenously. That such fixation results has been shown by other work from this laboratory (10). It is probably this steroid in the tissues which is responsible for the prolonged slightly higher excretion of 17-ketosteroids during the fifth to ninth days.

At the time when the greatest amount of nitrogen was being retained the excretion of 17-ketosteroids was within the range of the control period. It seems highly probable, therefore, that the 17-ketosteroids in the urine do not primarily represent the hormone actually involved in the anabolic process, but largely originate from excess amounts of circulating hormone to which is added a small fraction due to intracellular turnover in the target tissues.

Two interesting questions remain unanswered. If a high level of nitrogen storage can be obtained immediately by a single intravenous injection, could it be maintained by small intravenous or intramuscular doses thereafter? If the level of achieved nitrogen storage depends on the concentration of testosterone which penetrates the cells, what is the maximum rate possible? It is hoped that these questions will be answered by experiments now under way.

SUMMARY AND CONCLUSIONS

Single doses of 150–200 mg. of testosterone dissolved in human serum albumin solutions administered intravenously to normal young men led to a prolonged retention of nitrogen. Nitrogen retention was marked during the first twenty-four hours and reached its highest level on the second day after administration. There was no evidence of retention, however, during the first eight hours. The positive nitrogen balance continued at gradually decreasing levels for from eight to ten days.

When a dose of 25 mg. of testosterone was injected, no measurable nitrogen retention was observed. When a dose of 100 mg. was given, there was a slight retention of questionable significance. The response, therefore, does not seem to be proportional to dose, but appears to depend on achieving a sufficiently high level of hormone in the blood.

Retention of phosphorus and water accompanied the nitrogen storage but in the one case which was studied over the entire period, there was no evidence of change in sodium or potassium balance.

The level of testosterone in the blood fell rapidly after the intravenous infusion; at the end of one hour the amounts were at the limit of the analytic method. Very little α, β-unsaturated ketone was excreted in the urine. Within two hours approximately 40 per cent of the injected hormone was accounted for as excess 17-ketosteroids in the urine; in twenty-four hours 70 to 80 per cent was excreted in this form, mostly as a conjugate. There seemed to be a small secondary rise from the fourth to eighth days after injection.

Since nitrogen retention did not occur until from eight to nine hours after administration of the hormone, and was not proportional to the dose, it is proposed that the high blood level achieved for a short time by the intravenous administration leads to rapid penetration into tissue cells where it is bound in a form which is relatively slowly diffusible. That which remains in the blood and that which is taken up by the storage fat is rapidly metabolized to 17-ketosteroids. This first rapid conversion represents excess and not active material. The later small rise in 17-ketosteroids may represent the eventual metabolism of the active intracelluiar hormone.

Intravenous administration of testosterone dissolved in serum albumin solution appears to be a way of achieving relatively rapid nitrogen retention with a relatively high ratio of total nitrogen retained to testosterone administered.

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THE DISTRIBUTION OF I^{131} IN TISSUE OBTAINED AT NECROPSY OR AT SURGICAL OPERATION IN MAN*

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INTRODUCTION

RADIOACTIVE iodine, I^{131}, is receiving extensive trial in the diagnosis and treatment of thyrotoxicosis (1–11) and carcinoma of the thyroid (12–16) and has been proposed as an agent for the induction of myxedema in euthyroid cardiac patients with intractable angina pectoris and congestive heart failure (17–20). The biologic effects of radioactive iodine depend largely on the emission of beta rays; the interaction of gamma rays from the I^{131} within a particular organ contributes a relatively small per cent of the radiation effect at any one point (21), although total body radiation from gamma rays emitted by I^{131} in the thyroid may contribute additional radiation which at the present time is not accurately measurable. Since the range in tissue of the beta ray of I^{131} is only approximately 2 mm., the concentration of I^{131} within any organ determines in large measure the amount of radiation to that organ.

Despite the wide clinical use of radioactive iodine, data on the distribution of I^{131} in man and, consequently, on the radiation delivered to the thyroid and other organs, is scanty. Marinelli and Hill (22) have reported the concentration of I^{131} in the various tissues of a moribund patient with a thyroid adenoma who died five hours after the intravenous administration of 100 millicuries. The greatest concentration of I^{131} was in the stomach wall and contents; but thyroid, thyroid tumor, pancreas, and intestinal wall contained greater concentrations than blood. The I^{131} content of all other organs was comparable to blood, or less; the nervous system was noted to contain a particularly low concentration. More recently Rall *et al.* (23) have described the I^{131} distribution fifty-six hours after the oral adminis-

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tration of 63 millicuries to a patient with tracheal obstruction and recurrent episodes of stridor caused by a highly malignant thyroid carcinoma. During the fifty-six hours of life the patient excreted 69.1 per cent of the administered dose in the urine. At necropsy, normal thyroid tissue contained 20 per cent of the administered I^{131}, the highest concentration found in any organ. The liver, muscle mass, and marrow accounted for 3.9, 3.3, and 3.0 per cent respectively; other organs contained less. Trunnell *et al.* (24) have presented detailed data on the distribution of I^{131} in the tissues of 9 patients studied at necropsy. In 6 of the 9 cases surgical thyroidectomy or medical thyroidectomy with I^{131} had been previously accomplished. Thyroid, trachea, bile, spinal cord, adrenal and stomach contained the largest concentrations.

Since the available data are incomplete, particularly in subjects with normal thyroid glands, the present study comprising additional data on the distribution of I^{131} in man is reported.

MATERIAL AND METHODS

Thirteen cases were studied, 6 at necropsy; 7 were male and 6 female. The ages ranged from 51 to 73. In 5 of the 6 autopsy cases (*see* case histories at end of article) an aliquot of each organ was boiled in 2.5 N sodium hydroxide until digestion was achieved; the mixture was then diluted to a known volume with distilled water. One cc. of the diluted material was pipetted into glass cups; 1–2 mg. of sodium iodide and 2–3 mg. of gelatin were added and the mixture dried slowly under a lamp. Beta radiation was measured according to a method previously described (25); suitable correction was made for mass absorption. In the sixth case (Case 4) a weighed sample of tissue was minced and evenly distributed over the surface of an aluminum cup; sodium iodide and gelatin were added and the mixture dried and measured as described above. In 3 cases (Cases 1, 3 and 5) in which the thyroid gland was known to contain a large amount of I^{131}, the dilute digestion mixture was pipetted into special Marinelli beakers and the gamma radiation was measured (22).

The per cent of administered I^{131} in each organ was determined by comparison of the radiation from each organ with a standard. The standard consisted of an aliquot of the stock solution of I^{131} which had been administered to the patient, added to 2 N sodium hydroxide and digested muscle, and prepared and counted as above described. It was demonstrated in the following manner that no loss of I^{131} occurred during digestion and boiling. When I^{131} was added to previously digested muscle, the radiation was identical to that obtained when the same amount of I^{131} was added to muscle which was subsequently digested (26).

In the remaining 7 of the 13 patients, all euthyroid, 300 to 850 micro-

curies[1] of I^{131}, carrier-free, were administered four hours to seven days before orchidectomy or oophorectomy. Measurements of I^{131} content of the ovaries and testes were made by the method just described above.

Calculation of the per cent uptake and the equivalent roentgens delivered was made according to the following formulae (21):

$$1.\ \text{Per cent uptake} = \frac{\frac{\text{net counts in unknown}}{\text{wt. of aliquot}} \times \text{wt. of organ} \times \text{dilution of unknown} \times 100}{\text{dose administered} \times \text{net counts of standard} \times \text{dilution of standard}}$$

$$2.\ REP_B{}^{*2} = \frac{\text{microcuries administered} \times \text{uptake} \times 144}{\text{weight of organ}}$$

In Cases 1, 3 and 4 (Tables 2, 4, 5) and in the surgical specimens listed in Table 8, radiation is indicated directly by the approximate equivalent roentgens from beta rays delivered to each organ. In Cases 2, 5, 6 (Tables 3, 6, 7) calculation of equivalent roentgens delivered was not feasible; in these cases the uptake per gram of tissue as represented by net counts of I^{131} per gram is most indicative of the radiation received by each organ. The assumption was made in 12 of the 13 cases that no significant turnover of I^{131} occurred in each organ and that the biologic half-life was 8 days. The validity of this assumption is indicated by previous studies showing a biologic half-life of 7.5 days of I^{131} (physical half-life, 8 days) in the thyroid gland of euthyroid patients as measured by a 4-tube method of external counting (27, 28). In Case 1, where possible thyrotoxicosis existed, the biologic half-life of I^{131} in the thyroid gland was assumed to be 6 days (29). The REP_B delivered were calculated for the entire life of the isotope. Wherever possible the net counts per gram, the uptake of the total organ, and the REP_B delivered are compared to the values as measured for the thyroid gland.

The question was raised whether the I^{131} in the aliquot of each organ examined was representative of the distribution of I^{131} in the organ as a whole. In Case 4, the I^{131} radiation from each of several specimens of tissue from eight organs was measured (Table 1). Although the variation in net counts in several samples of the same organ was large in absolute terms, *e.g.*, muscle, spleen, lung (Table 1), it was not large enough to alter significantly the relative uptake of I^{131} in the organs in comparison to each other and particularly to the thyroid gland. The variation in uptake in the different specimens of thyroid gland was large; the I^{131} content in thyroid specimen III was four and three times respectively that of specimens I and

[1] The microcurie utilized in these studies corresponds to that of the U.S. Bureau of Standards.

[*2] One REP is defined as an amount of radiation (other than protons) which releases in tissue an amount of energy equal to the energy loss of one roentgen of gamma radiation absorbed in air.

TABLE 1. RADIOACTIVITY CONTENT OF MULTIPLE SPECIMENS OF TISSUE FROM THE SAME ORGAN. CASE 4

Organ	Specimen number	Net counts per gram of tissue
Thyroid*	I	2.63×10^4
	II	3.24×10^4
	III	1.1×10^5
Muscle	I	1,035
	II	1,961
	III	1,119
Lung	I	17,806
	II	12,900
Pancreas	I	1,345
	II	1,200
Kidney	I	4,128
	II	4,300
	III	4,233
Liver	I	1,767
	II	2,076
	III	1,915
Testis	I	3,270
	II	3,160
	III	3,290
Spleen	I	1,471
	II	2,299

* Counted 49 days after other organs were counted.

II (Table 1). This is of particular interest with regard to the therapeutic use of I^{131}, since calculations of the radiation delivered to the thyroid gland usually assume a uniform distribution of I^{131} throughout the gland. Whenever possible, in the other 5 of the 6 autopsy cases, the aliquot which was digested and of which the I^{131} content was measured, consisted of several pieces of tissue from different parts of the organ.

RESULTS

The results are presented in Tables 2–9. The data (Tables 2, 4, 5 and 9) indicate that the major portion of the radiation from the administered

TABLE 2. DISTRIBUTION OF I^{131} IN CASE 1

Organ	Net counts per min. per gram of tissue	Per cent of net thyroid counts	Uptake of administered dose by total organ %	Per cent of thyroid uptake	Delivered REP	Per cent of REP delivered to thyroid
Thyroid	4,800,000	100.00	47.20	100.00	6,100	100.00
Lung	56,800	1.20	1.70	3.60	104	1.70
Liver	27,800	0.58	1.10	2.20	51.4	.84
Kidney	43,700	0.90	0.40	0.90	73.5	1.20
Spleen	30,500	0.63	0.20	0.40	57.0	.94
Pituitary	36,300	0.75	0.0008		68.5	1.10
Adrenal	24,100	0.50	0.02	0.04	39.5	.65
Pancreas	94,300	2.00	0.20	0.40	158	2.60
Brain	4,700	0.10	0.20	0.40	9.4	.15
Muscle	22,900	0.50	9.50	20.00	40.6	.67
Blood clot	42,800	0.90	1.00	2.10		

TABLE 3. DISTRIBUTION OF I^{131} IN CASE 2

Organ	Net counts per min. per gram of tissue	Per cent of net thyroid counts
1. Normal thyroid	48,200	100.00
2. Metastatic carcinoma of thyroid to		
a. Lung	149	.32
b. Rib	127	.27
c. Vertebra	114	.24
d. Peribronchial lymph node	87	.18
e. Skull	42	.10
3. Tissue free of carcinoma		
a. Lung	248	.51
b. Uterus	119	.25
c. Kidney	97	.20
d. Ovary	92	.19
e. Spleen	76	.16
f. Liver	76	.16
g. Adrenal	40	.10
h. Muscle	40	.10
i. Pituitary	0	0

TABLE 4. DISTRIBUTION OF I^{131} IN CASE 3

Organ	Net counts per min. per gram of tissue	Uptake of administered dose by total organ %	Per cent of thyroid uptake	Delivered REP	Per cent of REP delivered to thyroid
Thyroid*	47,600	38.00	100.00	14,000.00	100.00
Bile	2,630	.028	.07		
Lung—2	936	.30	.80	.97	.007
Liver	1,325	.70	1.80	1.53	.011
Kidney—2	962	.08	.20	.93	.007
Spleen	625	.03	.08	.83	.006
Pituitary	640	.0001	.0003	.504	.0036
Adrenal—2	650	.005	.013	.72	.005
Pancreas	551	.018	.005	.61	.0044
Muscle	270	1.50	4.00	.302	.002
Gonad—1	702	.0019	.005	.78	.0056
Marrow	516				

* Thyroid gamma radiation was measured. Beta radiation was measured from all other organs.

TABLE 5. DISTRIBUTION OF I^{131} IN CASE 4

Organ	Net counts per min. per gram of tissue	Per cent of net thyroid counts	Uptake of administered dose by total organ %	Per cent of thyroid uptake	Delivered REP	Per cent of REP delivered to thyroid
Thyroid	7.7×10^6	100.00	40.00	100.00	28,800.0	100.00
Lung	15,350	0.20	4.20	10.50	81.7	.29
Liver	1,920	.025	.63	1.60	9.5	.034
Kidney	4,220	.05	.35	0.90	19.0	.066
Spleen	2,500	.03	.17	.45	13.9	.048
Adrenal	3,030	.04	.001	.0025	1.2	.004
Pancreas	1,270	.01	.03	.75	5.1	.018
Muscle	1,370	.01	5.00	12.50	6.0	.021
Gonad	3,200	.04	.02	.05	16.3	.057
Plasma	6,000	.078	3.80	9.40		

TABLE 6. DISTRIBUTION OF I^{131} IN CASE 5

Organ	Net counts per min. per gram of tissue	Per cent of net thyroid counts
Thyroid	650,000	100.00
Lung	359	.055
Liver	1,430	.20
Kidney	284	.04
Spleen	210	.03
Pituitary	173	.025
Adrenal	214	.03
Brain	21	.003
Muscle	32	.005
Gonad	169	.025
Marrow	500	.07
Bloot clot	196	.03
Stomach	279	.04

I^{131} was delivered to the thyroid gland. If the thyroid radiation is taken to equal 100 per cent, the other organs in every case examined received only a small fraction of the radiation delivered to the thyroid gland (Table 9), of the order of 1 per cent and usually less. In most instances, the organs in the order of decreasing I^{131} uptake per gram of tissue were lung, kidney, pituitary, liver, gonads, spleen, adrenal and pancreas (except for one high reading, Case 1). This order of distribution was not uniform in all cases.

Comparison of the data in Table 7 and Tables 2–5 indicates a slightly higher uptake of I^{131} in gonads obtained at necropsy as compared to those obtained at surgery. The uptakes in the ovaries varied from an unmeasurable amount in Cases 11 and 13 to .0019 per cent in Cases 3 and 12. The

TABLE 7. DISTRIBUTION OF I^{131} IN CASE 6

Organ	Net counts per min. per gram of tissue	Per cent of net thyroid counts
Thyroid	7,750	100.00
Lung	7	.09
Liver	42	.54
Kidney	8	.10
Spleen	5	.07
Pituitary	72	.90
Adrenal	10	.12
Muscle	5	.07
Gonad	8	.10
Marrow	220	2.80
Stomach	33	.40

TABLE 8. RADIOACTIVITY CONTENT OF GONADS OBTAINED AT OPERATION AFTER A TEST DOSE OF I^{131}

Patient, Age	Organ	Dose, microcuries	Date of dose	Date of operation	Uptake	REP delivered	Diagnosis
J.H. 50	Testis	850	3/30/48	4/ 1/48	Right: .001% Left: .003%	.09 .27	Carcinoma of prostate
B.H. 73	Testis	340	4/15/49	4/16/49	.011%	.44	Carcinoma of prostate
S.S. 72	Testis	320	10/12/49	10/13/49	.006% each	.25	Carcinoma of prostate
J.F. 69	Testis	300	9/30/49	10/ 1/49	Left: .009% Right: .006%	.32 .13	Carcinoma of prostate
M.F. 45	Ovary	850	4/ 5/48 8 a.m.	4/ 5/48 12 noon	No measurable amount		Fibroid uterus
L.O. 40	Ovary	850	4/ 7/48	4/ 8/48	.006%	.22	Ectopic pregnancy (left)
A.N. 67	Ovary	850	4/ 7/48	4/14/48	No measurable amount		Senile ovaries; carcinoma of left ovary

TABLE 9. SUMMARY

Organ	No. of observations	Per cent net thyroid counts	No. of observations	Uptake of administered dose by total organ, %	No. of observations	Per cent of thyroid uptake	No. of observations	Per cent of REP delivered to thyroid
Thyroid	6	100	3	38.0 –47.2	3	100	3	100
Lung	5	.05 –1.2	3	.3 – 4.2	3	.8 –10.5	3	.007 –1.7
Liver	5	.024– .58	3	.63 – 1.1	3	1.6 – 2.2	3	.011 – .84
Kidney	5	.04 – .9	3	.08 – .4	3	.2 – .9	3	.007 –1.2
Spleen	5	.03 – .63	3	.03 – .2	3	.08 – .45	3	.006 – .94
Pituitary	4	.025– .9	2	.0001– .0008	2	.0003– .0017	3	.0036–1.1
Adrenal	5	.03 – .12	3	.001 – .02	3	.0025– .04	3	.0026– .65
Pancreas	2	.10 –2.0	3	.001 – .02	2	.005 – .4	3	
Brain	2	.003– .1	1	.2	1	.4	1	.15
Muscle*	5	.005– .5	3	1.5 – 9.5	3	4–20	2	.002 – .67
Gonad	4	.025– .19	2	.0019– .02	2	.005 – .05		
Marrow	2	.07 –2.8						
Blood clot	2	.03 – .9						
Uterus	1	.25						
Plasma	1	.08						
Bile			1	.028	1	.07		
Stomach	2	.04 – .4						

* Muscle mass estimated to equal 15,000–20,000 Gm.

radiation to the testis was measurable in all instances; the uptake in the operative cases varied from .001 to .011 per cent of the test dose. In Case 4, in the testis obtained at autopsy the uptake was 0.02 per cent, the highest noted in the various gonads examined; in this instance marked scrotal and testicular edema had been present.

Bone marrow was obtained in 3 cases; in 1 instance (Case 3) the uptake relative to other organs and the thyroid was low. In Case 5 the bone marrow contained more I^{131} (net counts/minute/gram of bone marrow) than any other nonthyroid organ except liver. In Case 6, bone marrow studied eighty days after I^{131} administration, when the blood I^{131} level would be very low, contained more I^{131} than any other organ except the thyroid gland.

Four pituitary glands contained 0.004, 0.0025, 0.75 and 0.9 per cent per gram of tissue compared to the thyroid; a fifth pituitary gland contained no measurable activity.

Bile was studied in only 1 autopsy; the uptake four days after I^{131} administration was greater than in any other organ examined, except the thyroid (Case 3, Table 4).

DISCUSSION

Animal studies have shown the marked concentrating power of the thyroid gland for I^{131} as compared to other organs (30–33). Studies in man, utilizing external Geiger-Mueller counting techniques, have similarly indicated the avidity of the thyroid gland for I^{131}. Previous observations and our data on material obtained at necropsy and after operation in euthyroid subjects (Tables 7 and 9) are confirmatory of the high I^{131} uptake by the thyroid gland and the relatively low uptake by other organs. Although the cases studied by us were not strictly comparable to those of Trunnell *et al.* (24), the results of the present study are in general agreement. The uptake of 38 and 40 per cent by the thyroid gland in Cases 3 and 4 (both euthyroid) is in the high range of normal when compared to the uptake as determined by the 4-tube method of external counting (normal 29.5 ± 4.8 per cent (27, 28)). The differential preference for I^{131} by the thyroid gland is even more marked when the comparative data are considered in terms of "per cent thyroid REP delivered" or "per cent of thyroid net counts per gram of tissue." In the instance of the lung, liver, kidney, spleen, pancreas and muscle mass, the thyroid radiation is 100 to 2,000 times greater. All previous studies have shown an increased avidity of the thyroid gland for I^{131} in thyrotoxicosis. The differential between the thyroid and other organs would therefore be greater in patients with thyrotoxicosis than that demonstrated in the present study.

The present data permit calculation of the range of radiation delivered

by various doses of I^{131} to the organs of euthyroid patients. For example, a tracer dose of 100 microcuries, commonly in use for diagnostic purposes, would have delivered in Case 3 the following equivalent roentgens: thyroid (weight, 8.4 grams) 650; lung, .05; liver, .07; kidney, .04; pituitary, .014; adrenal, .036; and gonad, .074. Since there is no extensive body of evidence on the permissible exposure of the various organs to the beta radiation of I^{131}, the significance of these data are not yet apparent. Microscopic examination of the tissues in Cases 1 to 6 has revealed no evidence of injury clearly attributable to I^{131} except in the thyroid gland (34). Furthermore, our clinical experience during the past three to four years (which includes the administration of therapeutic doses of 1.7 to 35 millicuries of I^{131} to 150 patients with thyrotoxicosis, 17 to 206 millicuries to 50 patients with intractable angina pectoris or congestive heart failure, and 21 to 209 millicuries to 15 patients with carcinoma of the thyroid) is also consistent with the fact that relatively small amounts of radiation are delivered to organs other than the thyroid. Repeated examinations of the blood, urine and various liver function tests in the aforementioned 215 patients have revealed no adverse effects. It would, therefore, appear that radiation of the order of magnitude reported here is not immediately harmful to the kidney, liver or bone marrow. Trunnell (14), however, has demonstrated bone marrow depression following larger doses of I^{131}. Although the bone marrow uptake in Cases 5 and 6 of the present study was of high magnitude compared to other nonthyroidal organs, we have not observed bone marrow depression following total doses as large as 209 millicuries.

Duffy *et al.* (35) reported periods of amenorrhea in 3 premenopausal females after administration of large doses of I^{131}. A testicular biopsy specimen from a 50-year-old male similarly treated revealed atrophic changes and no evidence of spermatogenesis. Their studies demonstrated, however, no significant concentration of I^{131} in either the rat or human ovary and they concluded that ovarian function was not directly influenced by sublethal radiation. One of our patients, S. S., a 14-year-old boy, received 137 millicuries of I^{131} for a papillary adenocarcinoma of the thyroid with metastasis to the lung (15). Myxedema was induced and thyroid was administered. The subsequent development of sexual maturity in this patient suggests the absence of any immediate adverse effect of I^{131} on the gonads. This is consistent with the low uptake of I^{131} and the small amount of radiation delivered to the gonads, observed in the present study.

SUMMARY AND CONCLUSIONS

The distribution of I^{131} was studied in 13 patients. Seven euthyroid patients were given 300–850 microcuries of carrier-free I^{131}, from four to one hundred and sixty-eight hours before orchidectomy or oophorectomy. In

the remaining 6 cases, a dose of 1.7 to 39.1 millicuries was administered from one to eighty days before death. The data indicate that the major portion of the radiation from the administered I^{131} was delivered to the thyroid gland; other organs received only a small fraction of the radiation delivered to the thyroid, 1 per cent or less. In most instances the organs, in order of decreasing I^{131} uptake per gram of tissue, were lung, kidney, pituitary, liver, gonad, spleen, adrenal, and pancreas. Four pituitary glands contained 0.004, 0.025, 0.75 and 0.9 per cent per gram of tissue compared to thyroid; a fifth pituitary gland contained no measurable activity. In 1 case bone marrow contained amounts of I^{131} per gram equal to or greater than any organ except thyroid. In another instance only thyroid and liver contained greater radioactivity. The uptake in the ovaries was unmeasurable in 2 cases and was 0.0006 and 0.0019 per cent of the administered dose in another 2 cases. The radiation to the testis was measurable in all instances; the uptake in the operative cases varied from 0.001 to 0.011 per cent of the test dose. In Case 4, in which marked testicular edema had been present, the testis examined at necropsy contained 0.02 per cent. The above data indicating the relatively small amount of radiation delivered to organs other than the thyroid gland are consistent with our clinical experience in 215 cases treated with total doses of from 1.7 to 209 millicuries. Evidence of immediate injury to the bone marrow, kidney, liver or gonads has not been obtained following doses of this magnitude.

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CASE HISTORIES

Case 1, P.S., BIH #2986, a 73-year-old woman, was admitted because of congestive heart failure of two months' duration and pneumonia of three weeks' duration. During the previous year, there had been weight loss, fatigue, tremor, sweating, prominence of the eyes, and paroxysms of irregular heart action. On physical examination the patient appeared gravely ill and was in acute respiratory distress. The blood pressure was 170/100, the temperature was 102, the pulse was 140, and the respirations were 24. The skin was warm and moist; slight exophthalmos was present. There was a large substernal goiter, only the upper portion of the lobes and isthmus being palpable. The breath sounds were diminished and there were moist rales at both lung bases. The heart was enlarged; a loud apical systolic murmur was audible. The remainder of the physical examination was not remarkable. Following an oral tracer dose of I^{131}, net counts per minute 15 centimeters from the thyroid gland as measured by a single tube were within the range observed in patients with thyrotoxicosis (36). On November 7, 1948, a dose of 36 millicuries of I^{131}, carrier-free, was administered orally; urinary collections were not feasible. Because of the critical condition of the patient, the administration of saturated solution of potassium iodide orally (5 drops, twice daily), and sodium iodide (1 gram, intravenously) was begun fifteen hours later. The patient, however, failed to improve and expired thirty-three hours after receiving I^{131}. The pathologic diagnoses included: 1) involutional nodular goiter with substernal extension, 2) multiple pulmonary emboli, 3) bronchopneumonia, and 4) cardiac hypertrophy, dilatation, and coronary arteriosclerosis. The distribution of I^{131} is indicated in Table 2.

Case 2, J.S., BIH #M 329, a 69-year-old woman, entered the hospital critically ill with carcinoma of the thyroid and multiple skull metastases. On May 18, 1948, a test dose of 1.7 millicuries of I^{131} was administered orally; 50 per cent was excreted in the urine during the following seventy-two hours. The patient expired May 21, 1948, seventy-two hours after the administration of I^{131}. The pathologic diagnosis was papillary adenocarcinoma of the thyroid with widespread metastases. The distribution of I^{131} is indicated in Table 3.

Case 3, B.F., BIH #M 4944, a 69-year-old woman, was moribund following a cerebrovascular accident. A test dose of 2.1 millicuries of I^{131} was administered orally through a Levine tube; urinary collection was not feasible. The patient expired four days after the administration of I^{131}. The pathologic diagnoses included fresh massive cerebral hemorrhage, encephalomalacia, cerebral arteriosclerosis, bronchopneumonia, and multiple pulmonary artery thrombi. The distribution of I^{131} is shown in Table 4.

Case 4, D.C., BIH #93141, a 51-year-old man, entered the hospital critically ill with rheumatic heart disease, intractable congestive heart failure, and massive anasarca. On March 14, 1947, a dose of 17 millicuries of I^{131} was administered. Seven per cent was excreted in the urine during the following seventy-two hours; the total urinary output in that period was only 800 cc. The patient expired seven days after receiving I^{131}. Pathologic diagnoses were: healed subacute bacterial endocarditis, mitral stenosis and insufficiency, aortic stenosis and insufficiency, thrombosis of the left descending and right coronary arteries, fibrosis of the left ventricle, chronic passive congestion of the viscera, bilateral hydrothorax, central necrosis of the liver, and bronchopneumonia. The distribution of I^{131} is indicated in Table 5.

Case 5, L.S., BIH #M 1326, a 52-year-old man, who had suffered from angina pectoris for ten years, entered the hospital in status anginosus. All usual therapeutic measures having failed to ameliorate symptoms, I^{131} was administered for the purpose of inducing myxedema. On July 23 and 30, and August 10 and 26, 10, 10, 9.1 and 10 millicuries respectively of I^{131} were administered. The 72-hour urinary excretions were 74, 95, 88 and 86 per cent respectively. Hypometabolism was not induced and the patient continued to experience many attacks of precordial distress. He expired five weeks after the first dose of I^{131} and five days after the last dose. The pathologic diagnoses included: miliary ischemic necrosis of the left ventricle and interventricular septum, miliary infarctions of the left ventricle, myocardial fibrosis, coronary arteriosclerosis and post-irradiation thyroiditis. The distribution of I^{131} is indicated in Table 6.

Case 6, J.S., BIH #M 1834, a 54-year-old male, had had severe angina pectoris for the previous five months following an acute myocardial infarction. All customary treatment having failed, on June 6, 1948, a dose of 43 millicuries of I^{131} was administered orally for the purpose of inducing myxedema. The urinary I^{131} excretion was 50 per cent of the administered dose during the following seventy-two hours; the patient was known to have discarded several specimens of urine. Hypometabolism was not induced and he failed to experience relief of angina pectoris. Eighty days after I^{131} administration he suffered an acute myocardial infarction and expired. The pathologic diagnoses were: coronary arteriosclerosis with multiple occlusions, old infarct of the left ventricle, diffuse fibrosis of the myocardium, pleural effusion, atheromatous plaques of the aorta, focal acute pancreatitis and splenitis, post-irradiation thyroiditis and thyroid necrosis. The distribution of I^{131} is indicated in Table 7.

HORMONE STUDIES IN A CASE OF ADRENOGENITALISM DUE TO NEOPLASM OF THE ADRENAL CORTEX

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THE occurrence of large amounts of androgen in the urine of humans with adrenogenitalism resulting from adrenal cortical neoplasia is well known (1). Little is known concerning the hormone content of the tumors themselves. Except for the report of Slot (2), who found in the tumor of a patient with adrenogenitalism a "quantity of male and female hormone not more than in organs (for instance liver) of normal people," the authors are not aware of any attempts to correlate the hormone content of the tumor with that of the urine. The present study consisted of hormonal analyses of urine before and after operation and of the tumor removed at operation from a 7-year-old female with adrenogenitalism.

METHODS

Urinary and tumor extract measurements were made by the following methods, appropriately modified wherever necessary in the case of the tumor extracts: 17-ketosteroids by the method of Drekter, Pearson, Bartczak and McGavack (3); corticosteroid by the method of Lloyd and Lobotsky (4); pregnanediol by the method of Guterman and Schroeder (5); biologically active androgen by the 1-day-old chick method of Franck *et al.* (6); gonadotropin by the method of Lloyd, Morley, Morrow, Lobotsky, and Hughes (7); and dehydroisoandrosterone by the method of Allen *et al.* (8) and by the Pettenkofer reaction as modified by Munson, Jones, McCall, and Gallagher (9). Tumor cholesterol was determined by a modification of the method of Schoenheimer and Sperry (10). Paper chromatography of the urinary and tumor extracts was carried out by Dr. W. S. Haines of the Upjohn Company, employing a modification of the method of Zaffaroni, Burton, and Keutmann (11). Extraction of the tumor was performed by the method described subsequently, which was suggested by Dr. Haines.

CLINICAL AND PATHOLOGIC DATA

The patient was a 7-year-old girl who first showed the development of pubic hair one month before admission (Figs. 1 and 2). For several years previously the child had

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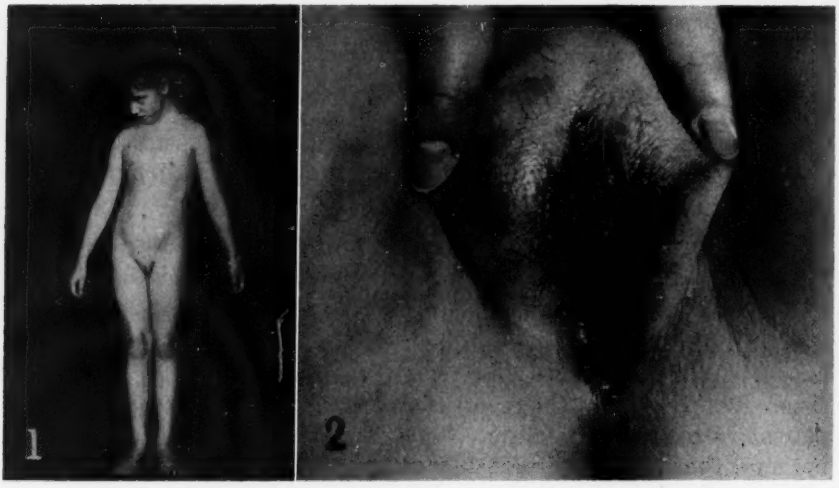

FIGS. 1 and 2. A 7-year-old girl with adrenogenitalism. The clitoris is enlarged and a moderate amount of pubic hair is present.

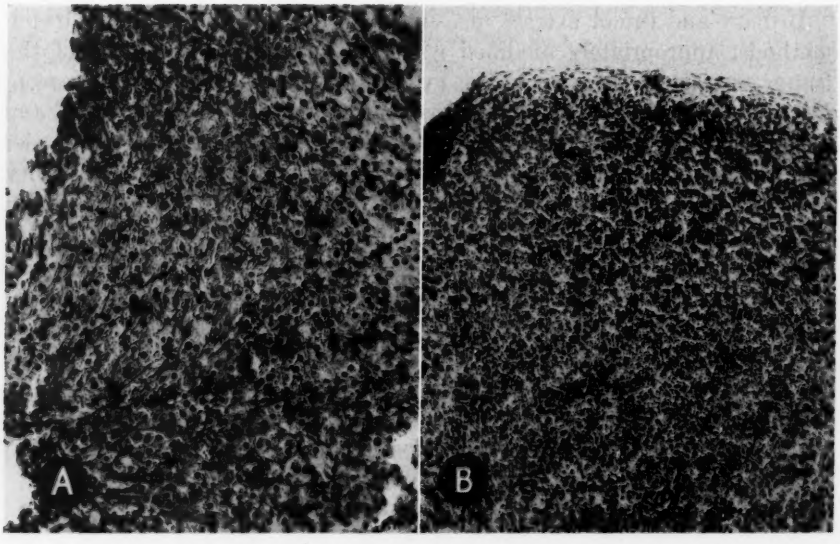

FIG. 3. (A) Frozen section of adrenal cortical tumor stained with Sudan III and hematoxylin. Note absence of sudanophilic material. ×155. (B) Normal rat adrenal prepared in the same manner. A large amount of sudanophilic material is present. ×155.

been somewhat larger than her coevals. Her general health had been excellent. Physical examination showed a symmetrically developed girl who was 8 cm. taller than the upper limits of normal for her age. There was a moderate amount of pubic hair, development of the labia minora was considerably greater than normal, and a clitoris measuring 8 mm. in diameter was found. Bone age was interpreted as being approximately that of a 10-year-old child. The sella turcica was normal. Routine hematologic examination and urinalysis were not remarkable. Serum sodium was 141 mEq./L. and serum potassium was 3.5 mEq./L. Eosinophil response to epinephrine was normal. A vaginal smear showed no estrogen effect. The results of oral and intravenous glucose tolerance tests were within normal limits. Intravenous and retrograde pyelography revealed a left kidney lower than normal. No definite mass could be seen. Preoperative and postoperative findings are discussed in detail in the following sections.

Operative and pathologic findings: At operation an encapsulated, soft tumor measuring approximately 4.5 to 5.0 cm. was found above the left kidney. The tumor was extremely soft and friable. Its weight was 48 Gm. Histologically the cell pattern was fairly uniform, consisting of sheets of cells separated only by relatively few capillaries. The cells were of medium size with slightly vesicular nuclei, varying considerably in size. The cytoplasmic structure in the majority of the cells was fairly dense and of a finely granular and reticular pattern. Many cells, however, scattered irregularly and without arrangement showed relatively clear cell bodies. This general cell structure is clearly shown in Figure 3, which also demonstrates the almost complete absence of sudanophilic material, even in the cells with relatively clear cell bodies.

CHEMICAL DATA

Extraction of the tumor: The tumor weighed 48 Gm. A block weighing 3 Gm. was taken for histologic studies. Within ten minutes after its removal the remaining 45 Gm. of the tumor was frozen with carbon dioxide ice. The tissue was pulverized with a mortar and pestle in the presence of sufficient carbon dioxide ice to keep the tissue frozen at all times. The powdered tissue was extracted for one hour at room temperature with six volumes of acetone. The supernatant fluid was removed and the residue re-extracted twice with 250 ml. of acetone. The acetone fractions were pooled and the acetone removed in vacuo. Sufficient water and acetone were now added to make the total volume 300 ml. of a 50 per cent water-acetone mixture. This was extracted with 300 ml. of petroleum ether and emulsions broken by freezing and centrifugation. The petroleum ether fraction (Fraction 1) was washed once with acetone and water. The acetone-water fractions were pooled and the acetone was removed in vacuo. The aqueous residue was

TABLE 1. TUMOR ANALYSIS

	Fraction I	Fraction II
Cholesterol, 1.2 mg./100 mg. (total 585 mg.)	585 mg.	0
Corticosteroid (formaldehyde-forming lipid-soluble material)	350γ HCHO 3.85 mg. desoxycorticosterone equivalents	76γ HCHO 0.836 mg. desoxycorticosterone equivalents
17-Ketosteroids	0	0
Biologically active androgen (as androsterone)	less than .125 mg. total	less than .125 mg. total

extracted five times with ethylene chloride, equal volumes. The ethylene chloride was washed with sodium hydroxide and water, which were in turn washed back with ethylene chloride. The ethylene chloride fractions were pooled to form Fraction II.

Analysis of the tumor: Analysis of the tumor is summarized in Table 1. The cholesterol concentration was low, although the total amount of cholesterol present was considerable. The content of formaldehyde-forming lipid-soluble material was also considerable. This material has been called "corticosteroid" since this group of steroids is the only one known to be in adrenal tissue, which will form formaldehyde upon oxidation with periodic acid. However, it is realized that the possibility of unknown formaldehyde-forming

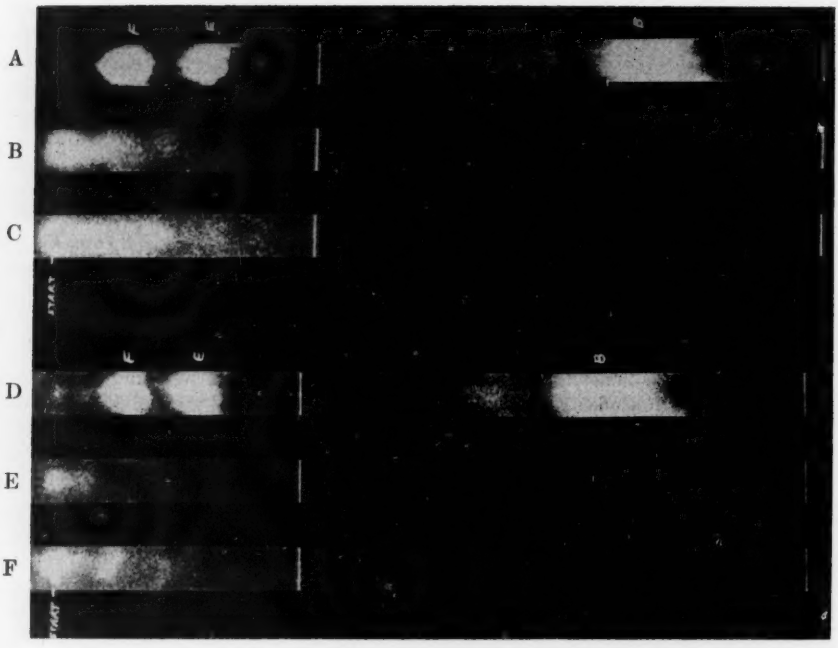

FIG. 4. Papergram of the urine and tumor residues: A and D, standard solutions; B and C, urine residues; E and F, tumor fractions I and II, respectively.

lipid-soluble materials exists. No detectable 17-ketosteroids or biologically active androgen were found in either fraction of the tumor extract.

A photograph of the papergram (12) obtained when urine and tumor residues were chromatographed is shown in Figure 4. On strips A and D were placed 20γ each of corticosterone (compound B), 11-dehydro-17-hydroxy-corticosterone (compound E), and 17-hydroxycorticosterone (compound F). On strips B and C were placed 50γ of corticosteroid from urine (formaldehyde value × factor of 11). Strips E and F are those on which were placed amounts of residue containing 50γ of corticosteroid from Fractions I and II, respectively, of the tumor.

The urinary residues moved to a position in the region of that of compound F, and there is a suggestion of movement towards the position occupied by compound E.

There was no movement of the residue from tumor Fraction I. However, there was a distinct, although slight, movement of the residue of Fraction II to occupy a position in the region of that of compound F, and there was a shadow of material which was intermediate in polarity between compound F and compound E.

Urinary hormone excretion: Urinary 17-ketosteroid and corticosteroid values are shown in Table 2. Preoperatively, an extremely large amount of 17-ketosteroid and about twice the normal amount of corticosteroid were excreted. Postoperatively the levels of both of these materials fell to the normal range. For a few days immediately after operation the 17-ketosteroid excretion decreased to a low level, suggesting that inhibi-

TABLE 2. 17-KETOSTEROID AND CORTICOSTEROID EXCRETION

	17-Ketosteroids, mg./24 hrs.	Corticosteroid, mg. formaldehyde/24 hrs.	
		Total	Water-soluble
Day before operation			
16	69.3		
15	82.0		
14	71.6	36	
9	77.6		
8		35	25
3		27	26
Day after operation			
1	4.4		
4	1.8	15	15
9	0.6	11.3	
67	5.6	18.6	
95	3.5		

tion of formation of 17-ketosteroid precursors had occurred. On the ninth preoperative day, when the 17-ketosteroid excretion was 77.6 mg. per twenty-four hours, biologically active androgen equivalent to 2.35 mg. of androsterone was present in the urine. Dehydroisoandrosterone excretion as measured on a urinary residue obtained following acid hydrolysis of the urine was 10 mg. when measured by the reaction described by Allen, and 16.5 mg. when estimated by the Pettenkofer reaction. On the fourteenth preoperative day, when the 17-ketosteroid excretion was 71.6 mg. per twenty-four hours and the corticosteroid excretion was 36 micrograms of formaldehyde, 96.2 mg. of pregnanediol as determined by the Guterman procedure was found. Postoperatively none of this material was found in the urine. On two occasions preoperatively and one postoperatively, no gonadotropin of either the uterine-weight-increasing or the ovarian-hyperemia-producing type was detectable.

DISCUSSION

Mason of the Mayo Clinic (13) has extracted 5 tumors of the adrenal cortex, all of which were associated with the development of Cushing's

syndrome. In general his findings are similar to those reported for our patient. 17-Ketosteroid content was from 0 to 2.68 mg. One tumor contained considerable estrogenic activity. In 2 tumors total corticosteroid values of 1.85 mg. and 7.76 mg. were found and another tumor contained 0.6 mg. of water-soluble and 0.5 mg. of benzene-soluble corticosteroid.

The tumor causing the high androgen levels which resulted in our patient's virilization was probably composed of very actively secreting tissue as indicated by its histologic characteristics and low cholesterol content. The large amounts of steroid which were found in the urine also indicate a high secretory rate. The clinical picture of adrenogenitalism without alteration in carbohydrate metabolism and the high urinary levels of 17-ketosteroids and of biologically active androgen suggest that there was an excessive production of androgen rather than glycogen-depositing steroids. However, no androgen was found in the tumor.

These data suggest three possible explanations: 1) the tumor was forming both androgen and cortical steroids but the rate of secretion was such that all of the androgen formed was immediately secreted, whereas some cortical steroids remained to be found on analysis; 2) the cortical steroids found in the tumor may have been precursors of androgen which was formed in the tumor but secreted rapidly from it; 3) only cortical steroids were formed and secreted by the adrenal and part of these were converted to androgen at some distant site such as the liver.

The frequently observed production of stigmata of excessive androgen levels and the increased excretion of 17-ketosteroids following administration of cortisone to humans demonstrate that cortical steroids can be converted to androgen. This observation tends to support the third hypothesis. However, if it can be assumed from the chromatographic identification that the tumor was secreting principally 17-hydroxycorticosterone (compound F), it is hard to reconcile the suggestion of conversion of this material into the large amounts of urinary 17-ketosteroids excreted by this patient, with the failure of exogenous compound F to increase urinary 17-ketosteroids as reported by Sprague, Power, and Mason (15). It is also difficult to explain the failure to develop signs of increased levels of carbohydrate-active cortical steroids on the basis of the theory that these steroids were secreted into the circulation in large amounts before being converted to androgens.

The demonstration of a material in the urine and in the tumor which, on chromatography, moves into a position in the region of that occupied by 17-hydroxycorticosterone does not offer positive proof of the identity of the substance, but it is very suggestive that this substance is 17-hydroxycorticosterone. This evidence carries considerably more weight in light of the demonstration that compound F (17-hydroxycorticosterone) is present in

animal adrenals (Kuizenga *et al.* (16, 17) and Haines and co-workers (18)) and that this substance is formed by the adrenal gland of the dog in vivo (Nelson *et al.* (19)) or by the adrenal of the cow in vitro (Hechter (20)). The demonstration of a substance which probably is compound F in adrenals of human origin adds another species to the list of those which produce this steroid.

A complete identification of the urinary 17-ketosteroids was not practicable. However, the Pettenkofer reaction as modified by Munson, Jones, McCall and Gallagher and the reaction described by Allen are considered by these authors to be specific for dehydroisoandrosterone or its derivatives. The comparison of these reactions was carried out on a urinary residue obtained following acid hydrolysis of the urine. The finding that 65 per cent more of the steroid gave the Pettenkofer reaction than the Allen reaction bears out the statement of Munson *et al.* that the Pettenkofer reaction measures derivatives of dehydroisoandrosterone which were formed during hydrolysis, such as Δ^5-3(β)-chloroandrostenone-17 and androstadieneone-17, as well as dehydroisoandrosterone itself. The reaction described by Allen apparently measures dehydroisoandrosterone alone. The nature of the 17-ketosteroids other than dehydroisoandrosterone excreted by the child in our study is undetermined, but it is apparent that very little androsterone was excreted, since the total biologic activity expressed in terms of androsterone was 2.35 mg. per twenty-four hours, which is only 3 per cent of the total 17-ketosteroids.

SUMMARY

1. A 7-year-old female child with adrenogenitalism resulting from an adrenal cortical neoplasm is described.

2. Before operation, she excreted from 69 to 82 mg. of 17-ketosteroids per day. On one day when the 17-ketosteroid excretion was 77.6 mg. per twenty-four hours, she excreted biologically active androgen equivalent to 2.35 mg. of androsterone, and dehydroisoandrosterone in amounts of 10 mg. when measured by the reaction described by Allen or 16.5 mg. when estimated by the Pettenkofer reaction.

3. On one occasion she excreted 96.2 mg. per twenty-four hours of a material reacting in a manner similar to pregnanediol. Corticosteroid excretion was from 27 to 36 gamma of formaldehyde in twenty-four hours, which is approximately twice the usual amount for children of this age. No gonadotropin was found on two occasions.

4. The tumor removed at operation weighed 48 grams. Histologically it showed almost no sudanophilic material. The cholesterol concentration was 1.2 mg. per 100 mg. Material yielding formaldehyde to an amount of 350 gamma when oxidized with periodic acid was present in the petroleum-

ether portion of the tumor extract and 76 gamma of formaldehyde was found by oxidation of the ethylene-glycol fraction. Essentially, neither 17-ketosteroid nor biologically active androgen was found in the tumor.

5. Paper chromatography of tumor and urine residues demonstrated in both a material which moved into the position occupied by compound F.

6. Postoperatively, 17-ketosteroid and corticosteroid excretion remained within the normal range.

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THE EFFECT OF ACTH IN A CASE OF CEREBROMACULAR DEGENERATION (TAY-SACHS DISEASE): AN ENDOCRINOLOGIC STUDY

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THE usual course of a patient with cerebromacular degeneration (Tay-Sachs disease, amaurotic family idiocy) is progressive cerebral degeneration and death. Morphologic and biochemical studies of postmortem material have been described (1, 2, 3). However biochemical studies during life and the endocrinologic aspects of the condition have received scant attention. Marburg (4) described morphologic changes in the endocrine glands in cases of Tay-Sachs disease and cited the findings of earlier workers. No functional endocrine studies, however, were reported.

Amaurotic family idiocy is believed to be related to the metabolic disorders classified as the lipoidoses. According to Globus (1) the basic derangement is a defective cell which is incapable of assimilating lipoid material, due perhaps to deficiencies in lipolytic enzymes.

The frequency of elevated serum cholesterol values in Cushing's syndrome and low values in Addison's disease is well known. The recent work of Conn (5) and Aldersberg (6) point to a close relationship between the adrenal cortex and lipoid metabolism.

With the above considerations, biochemical and endocrinologic studies of a case of cerebromacular degeneration were undertaken.

CASE REPORT

The patient, a white female child 18 months of age, was admitted to the Beth-El Hospital. Mental retardation and loss of vision were noted.

The child, the first, of parents of Hebrew extraction was born after a normal gestation and delivery, weighing 7 pounds 12 ounces and measuring 20 inches in length. There was no history of consanguinity. The mother was Rh negative, age 22. The father's age was 24.

Progress from birth was normal until the age of 7 months, when muscular hypotonia was noted. However, the child sat up when supported. The left leg was held in a position characteristic of congenital dislocation of the hip but no abnormality was demonstrated by x-ray examination.

At 8 months of age there was definite inability to sit up, hypotonicity was more marked and vision apparently poor. Examination of the ocular fundi at that time re-

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vealed the "cherry red" spot characteristic of cerebromacular degeneration and early optic atrophy. There was progressive deterioration until 15 months of age, the condition then remaining stationary until admission to the hospital.

Physical examination: On admission marked muscular hypotonicity was evident with inability to hold the head up, sit or stand. The weight was 23 pounds 6 ounces and the length 32 inches. The pulse was 150/min. and the temperature 99° F. The nutritional state was good. The head circumference was 19¼ inches and the fontanelles closed. The pupils were round and equal and reacted sluggishly to light. Eyes moved conjugately with frequent nystagmus. The eyes did not follow moving objects. Examination of the ocular fundi confirmed the original observations. However, the optic atrophy had progressed.

The heart sounds were rapid with no audible murmurs. The lungs were clear to auscultation and percussion. No abdominal organs or masses were palpable.

Muscular atrophy was absent but tonus was low. No grasp reflex was elicited. The Babinski reflex was positive bilaterally, eliciting clonic movements. A "startle" reaction to sudden sounds was present.

Therapy: After hematologic, biochemical and endocrinologic data were obtained during a control period, ACTH[1] was administered for a period of twenty-four days; the daily dosage ranged from 25 to 50 mg.

METHODS

Routine determinations were performed by procedures generally accepted as standard. Lipid phosphorus was determined by the method of Youngsburg (7); cholesterol by the Schoenheimer-Sperry method (8). The method of Natelson (9) was used for urine and serum citrates. Urinary estrogens were determined fluorometrically (10) and the 17-ketosteroids (11) and formaldehydogenic corticoids (12) were determined colorimetrically. The direct eosinophil counts were performed according to the method of Randolph (13).

DISCUSSION

No definite clinical changes were observed during the period of treatment. Except for a questionable increase of the hair on the thighs, no signs of overdosage were observed. There was a weight increase from 23 pounds to 23 pounds 13 ounces in ten days. During the period of reduced dosage of ACTH there was a weight loss of 6 ounces. With an increase in dosage the child gained 12 ounces, but despite continued therapy the weight continued to fall so that on the last day of treatment the child weighed 21 pounds 14 ounces. The blood pressure remained unaltered during therapy.

During the control period the serum lipid phosphorus (Fig. 1) was markedly elevated. During ACTH administration there was a fall of approximately 60 per cent, with a return to the high level following cessation of therapy.

Serum cholesterol, initially in the normal range, remained relatively

[1] Armour & Co., Chicago, Ill.

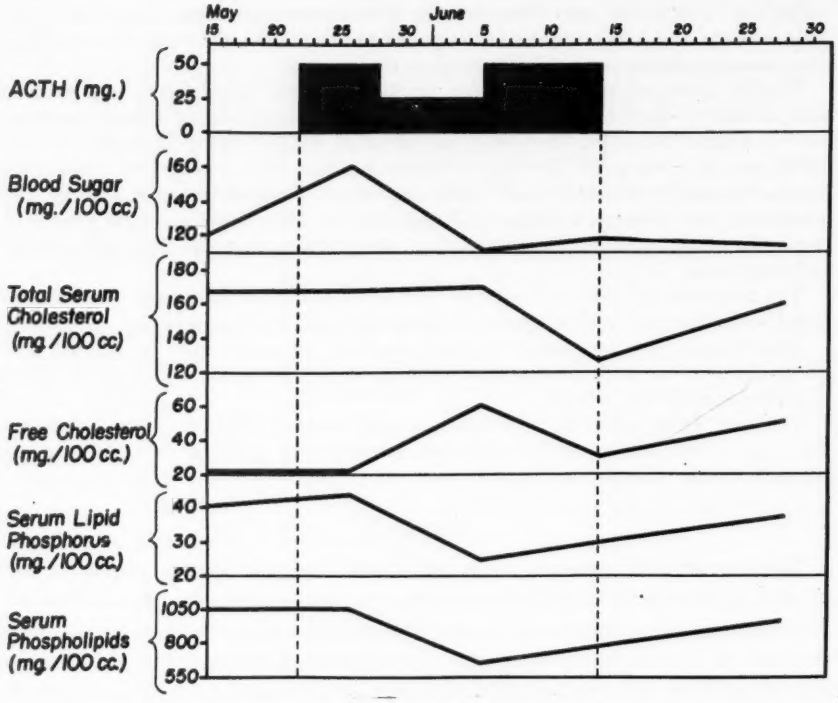

FIG. 1. The effect of ACTH administration on blood lipids and glucose in a case of Tay-Sachs disease.

constant for most of the treatment period and then dropped approximately 27 per cent. Following cessation of therapy there was a prompt reversal. There was no significant change in the percentage of the esterified fraction. This is in partial agreement with Cohn *et al.* (5) who demonstrated a fall of total and esterified serum cholesterol on administration of ACTH to normal individuals and to patients with Cushing's syndrome. However, Adlersberg (6) demonstrated only minor changes.

In Figure 2 the urinary excretion values for estrogen, 17-ketosteroids and formaldehydogenic corticoids are presented. The initial values were within normal range. During stimulation by ACTH they were markedly elevated. A rather prompt drop toward the initial levels occurred after cessation of therapy. The sharp rise in urinary estrogen is interesting. Since the ovaries are nonreactive at this age, one would assume the adrenal cortex to be the source of this steroid.

The blood glucose rose to hyperglycemic levels during the early phases, only to return to normal despite continued therapy (Fig. 1).

Study of the formed elements of the blood revealed an eosinopenia, leukocytosis and lymphocytosis in response to ACTH. The lymphocytosis was unexpected because one would expect a lymphopenia in view of the response of the eosinophils and total leukocytes to increased secretion of adrenal steroids.

Recent work in this laboratory demonstrated a decrease in serum and urine citrates during ACTH or cortisone therapy (14). This effect is clearly demonstrated in Figure 3.

The changes in urinary excretion of hormones, blood glucose, and number of circulating eosinophils are indicative of a normally reactive adrenal cortex. In this particular case, therefore, disturbance of adrenal cortical function may be excluded as an etiologic factor.

It is not known whether an elevation of serum phospholipids is of etiologic significance in Tay-Sachs disease. In view of the marked decrease of the level of these substances during treatment with ACTH, a trial of protracted therapy would be of interest.

Re-examination of the child four months following cessation of therapy

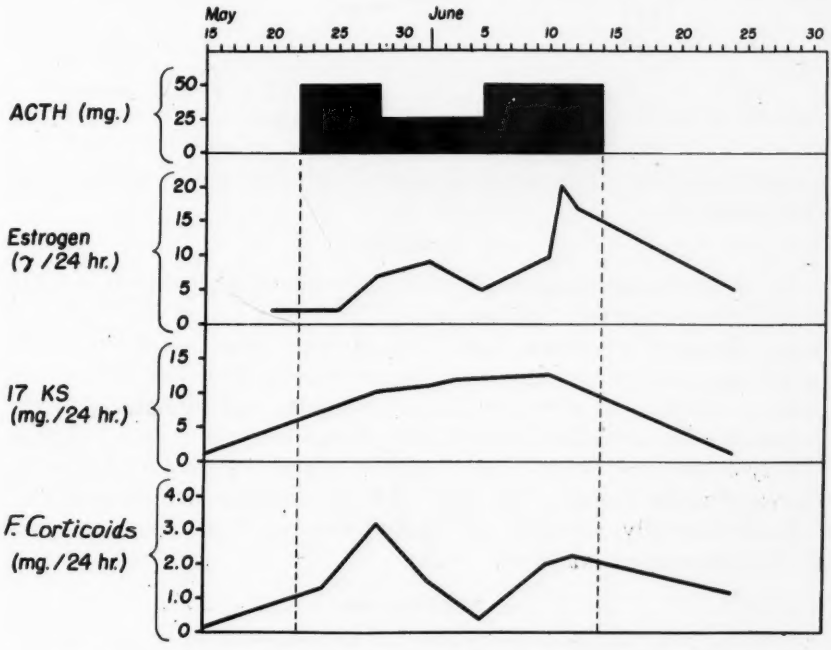

FIG. 2. The effect of ACTH administration on urinary excretion of steroids in a case of Tay-Sachs disease.

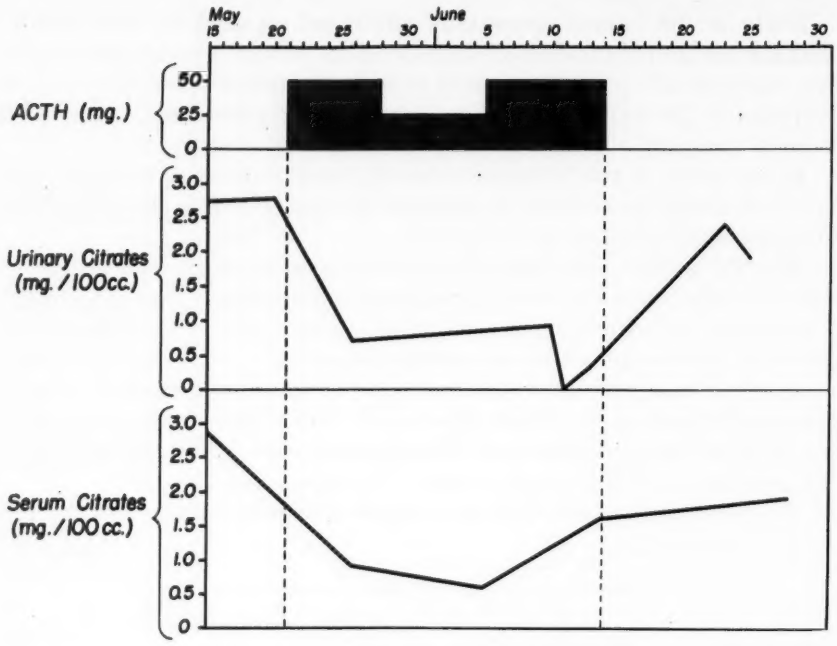

FIG. 3. The effect of ACTH administration on serum and urinary citrates in a case of Tay-Sachs disease.

revealed progressive deterioration, with the development of spasticity and hydrocephalus.

SUMMARY

An 18-month-old female child with cerebromacular degeneration (Tay-Sachs disease) received 1,000 mg. of ACTH over a period of twenty-four days in doses of 25 to 50 mg. daily. The adrenal cortex reacted normally, as demonstrated by hyperglycemia, eosinopenia, leukocytosis, and elevated urinary excretion of estrogens, 17-ketosteroids, and formaldehydogenic corticoids. The initially elevated lipid phosphorus was reduced approximately 60 per cent. Serum cholesterol, initially at a normal level, was also decreased under therapy. The blood and urine citrates were markedly depressed. Clinically, no significant changes were observed. The usual course of the disease was not altered.

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ACUTE AND CHRONIC ADRENAL INSUFFICIENCY IN A PATIENT SHOWING POLYGLANDULAR DEFICIENCY AND ACROMEGALY

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THIS patient presented such unusual clinical features that the case merits reporting and discussion. The occurrence of acromegaly (inactive or "burnt out"), and acute adrenal insufficiency is a rare combination to which there is scant reference in the literature (1, 2, 3, 4).

CASE REPORT

W.D. was a 41-year-old, unmarried, white, lumbermill foreman, who entered the hospital on February 17, 1949, in a state of semicoma. He had been well until forty-eight hours prior to admission when severe prostration developed and he lost his appetite. On the day of his admission he became irrational and semicomatose and had diarrhea. The past history later obtained from the patient and from members of his family was as follows:

In 1932 he consulted a physician because of undue fatigability and was told that he had heart disease. At that time he had no other symptoms and the fatigability disappeared spontaneously. About 1936 there was loss of axillary hair and partial loss of pubic hair. His hat size (7½) had not changed. However, his family believed that about ten years previously his jaw had become more prominent and his hands larger. His brother believed that the patient had failed to regain his full strength following a severe sore throat approximately in 1938. After he entered the Army in November 1942, he was unable to keep the pace during road marches and was hospitalized and discharged from the Army with a diagnosis of anemia. Following his discharge from the Army he had three episodes characterized by prostration and diarrhea precipitated by respiratory infections from which he spontaneously recovered in three to four days. During the third of these attacks he was taken to Camp Shoemaker Naval Hospital, but on admission there in February 1946, his acute symptoms had subsided. Libido was decreased, but the patient stated that he was able to have normal intercourse. Since 1942 he had been unable to perform any heavy physical exertion.

On admission to the hospital he was semicomatose, markedly dyspneic, cyanotic and moribund. His temperature was 106°, pulse 100, respirations 40 and blood pressure 90/60. During the first twelve hours the blood pressure fell to lower levels. There were rhonchi and sibilant rales in the chest and the heart tones could not be heard. There was evidence of marked dehydration and there was a persistent non-bloody diarrhea. The skin was dry and fawn-colored, and there was moderate obesity of the trunk. The mandible was prominent and there was an overbite of the lower teeth. The hands were

broad and the fingers short and heavy but not definitely abnormal. There was slight but definite increased pigmentation of some small scars on the legs and on the back, but otherwise there was no abnormal pigmentation. The prostate was small, being 50 per cent of normal size. There was no axillary hair and no body hair. The pubic hair was sparse and had a feminine distribution. The external genitalia were within normal limits. The arm span was 77 inches, height 71 inches, crown to pubis 35 inches, and pubis to soles of feet 36 inches. There was a moderate dorsal kyphosis (Fig. 1).

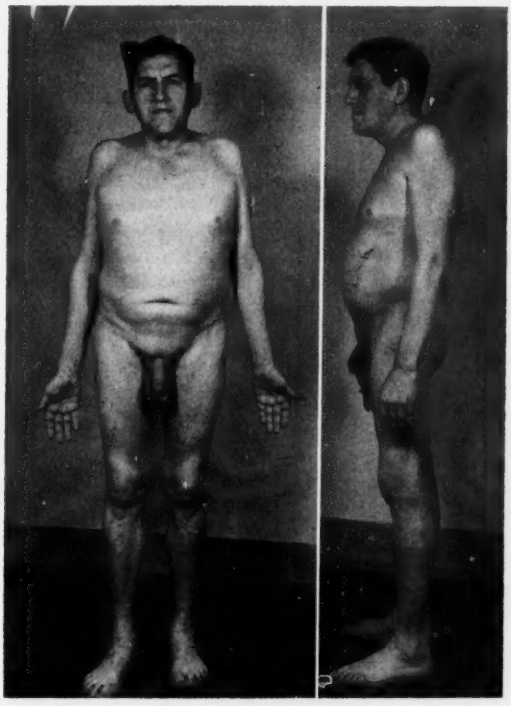

FIG. 1. Patient W. D. Photographs taken March 15, 1949.

Blood chemistry values were within normal limits for the most part, probably because of the vigorous treatment with salt replacement and adrenal cortical extract which was initiated upon admission. The laboratory values are noted in the summary of findings in Table 1.

Skull films showed a definite enlargement of the pituitary fossa, 22 mm. in A–P diameter and 12 mm. in depth; the anterior clinoids were sharpened, the dorsum thin, and the floor depressed into the sphenoid sinus. X-ray examination showed the following: in the hands the tufts of the terminal phalanges were prominent; in the mandible the angle was greater than normal and there was mild prognathism; in the right mid-lung field during the early part of his hospitalization there was a patch of pneumonic infiltration, which subsequently cleared completely.

Electrocardiograms taken during the course of his acute illness showed rhythm altera-

tions varying from auricular fibrillation to normal, transient low voltage and prolongation of the QT interval.

Histologic sections from a testicular biopsy specimen showed a marked decrease in the number of spermatogenic elements and only early-dividing forms and Sertoli cells were seen. The pathologic diagnosis was atrophy of interstitial tissue and maturation arrest.

The working diagnosis was acute adrenal insufficiency. During the first twenty-four hours he was given 6 liters of parenteral fluid and 500 cc. of plasma. The fluid consisted of dextrose in physiologic saline and of dextrose in distilled water. The amount of salt included 45 Gm. during the first twenty-four hours and subsequent smaller amounts.

TABLE 1. SUMMARY OF LABORATORY DATA

Date	RBC, million	Hgb., Gm./ 100 cc.	Serum K, mEq./L	Serum Ca, mg./ 100 cc.	Serum P, mg./ 100 cc.	Serum phosphatase, King-Armstrong units		Plasma CO_2, mEq./L	Serum* chloride, mEq./L	Blood urea, mg./ 100 cc.	Blood creatinine, mg./ 100 cc.	Blood glucose, mg./ 100 cc.	Serum cholesterol, mg./ 100 cc.	Serum protein-bound iodine, γ%	Basal metabolic rate, %
						H+	OH−								
2/17/48	4.9	14.0						18.0		12.0					
2/18	3.9	10.5	4.1						107			94	224		
2/19								18.9	110	28.3					
2/20	3.1	9.5						22.8	95	31.4	4.5	115			
2/24	2.4	7.5								25.5	2.75				
2/27	3.9	10.5						28.5	112	18.0	2.0				
3/ 6				10.5											
3/ 8										150	1.0				
3/ 9	3.0	9.5			3.75	1.5	5.6						210		
3/10														3.0	
3/11															−24
3/16				10.9	3.9										
3/19			5.0		3.6			26.9	101						
3/20				10.2	3.5										
3/29	3.4	11.0													−32

* Serum sodium determinations were not available at this time.
Serum protein, 6.25 Gm./100 cc.; albumen, 4.01; globulin, 2.21.
Glucose tolerance test: Fasting blood sugar, 70 mg./100 cc.; 1 hr., 64; 2 hrs., 40; 3 hrs., 38; 4 hrs., 58; 5 hrs., 58.
Urinary FSH—less than 5 mouse units.
Urinary 17-ketosteroids—2.6 mg./24 hrs.

His urinary output in the first twenty-four hours was approximately 100 cc., but it returned toward normal during the second twenty-four hours. During the first twenty-four hours of his hospitalization he was given 160 cc. of aqueous adrenal cortical extract (Upjohn) and he was then given 10 cc. of aqueous adrenal cortical extract every hour. During the first twenty-four hours the dosage of aqueous adrenal cortical extract was temporarily decreased, with a consequent deterioration in his condition. He improved when the previous dosage was again given; however on the sixth day of his hospitalization when the dosage was again temporarily decreased he became weaker. The full dosage was therefore continued and on the ninth hospital day lipo-adrenal cortical extract (Upjohn) was substituted. This substance was given intramuscularly, 4 cc. every four hours, until he was out of crisis; it was then gradually decreased to 1.0 cc. daily. With fluid replacement, marked anemia was evident and he was given 1,000 cc. of whole blood.

His response to therapy was gradual and after the first week there was steady improvement, with cessation of the diarrhea, the appearance of mental clarity, and gradual increase in strength.

A small dose of thyroid was administered, which was increased to 45 mg daily. He was maintained on lipo-adrenal extract, 1 cc. daily; supplemental sodium chloride, 3 Gm. daily; and methyltestosterone, 20 mg. daily. He improved remarkably, showed increased physical endurance, and was able to perform hard labor. There was also marked increase in pubic hair, increased length of the external genitalia, increased libido, and one to two erections with ejaculations per week. The prostate remained atrophic. The patient maintained his blood pressure at 110/78. Re-evaluation on October 21, 1948, revealed a serum phosphorus level of 4.8 mg./100 cc. The blood count had improved to within normal limits. A testicular biopsy showed no improvement. The basal metabolic rate had risen to the normal range. Lipo-adrenal extract was omitted and he was given desoxycorticosterone acetate linguets, 2 mg. twice daily.

His status was re-evaluated in May 1949, at which time it was thought that the hands were slightly increased in size. The serum phosphorus was elevated to 5.1 mg./100 cc. and the sella turcica appeared to be slightly larger than on initial examination; however, the visual fields remained normal. He was seen in consultation by Dr. Laurance Kinsell, who felt that the patient had suffered from a significant degree of myxedema, and that the striking improvement in the state of his adrenal insufficiency was far in excess of that which would have been anticipated with replacement therapy. It was thought that by raising the metabolic rate with thyroid medication, actual functional improvement in the state of the adrenals may have been produced. It was noted that the elevated serum phosphorus and the slight increase in the size of the sella could possibly mean a reactivation of the acromegaly which had been in the "burnt out" phase. The thyroid medication was therefore increased to 180 mg. daily and 750 mg. of testosterone propionate was implanted. This was done because the oral methyltestosterone would be ineffective in suppressing the pituitary—though its effect on the secondary sexual characteristics, aside from the prostate, was striking.

In June 1949, the patient was readmitted to the hospital suffering from what was believed to be a mild form of heat stroke. His blood chemistry was not remarkable and he recovered promptly.

In August 1950, a Thorn test was performed, with the following results: total fasting eosinophil count, 300 cells; total eosinophils four hours after administration of 20 mg. of ACTH, 250. This was interpreted as showing no evidence of adrenal cortical activity. The serum chloride, sodium and potassium were quite normal. The radioactive iodine uptake following administration of 120 microcuries of I^{131} showed no uptake in three hours and in twenty-four hours. Urinary 17-ketosteroids were 3.3 mg. per twenty-four hours. Glucose tolerance was as follows: fasting, 77; two hours, 58; three hours, 68; and four hours, 66 mg. per 100 cc. The same maintenance therapy was continued and at the date of writing the patient has done remarkably well.

COMMENT

The acromegalic appearance of the patient and the enlargement of the sella turcica indicated that some time in the past the patient had had an adenoma of the anterior pituitary. It is believed the present findings are due to the fact that at some time the adenoma was destroyed by some process such as hemorrhage and subsequent fibrosis, and the anterior pituitary became hypoactive. From the literature, depressed pituitary function appears to be fairly common following cessation of the hyperactive stage of acromegaly (1, 2, 5, 6, 7).

Atkinson, who performed the tremendous task of collecting all known cases of acromegaly throughout the world (3, 4, 7) found the rare combination of adrenal insufficiency with acromegaly. Thompson (6) stated that Addison's disease is sometimes caused by atrophy of the adrenal cortex as a result of inadequate stimulation by the anterior pituitary, but did not mention specifically that it might occur in acromegaly. Bernstein (8) of Stanford University reported a case of diabetes mellitus followed by Addison's disease and hypothyroidism, simulating panhypopituitarism, which at autopsy showed preservation of testicular structure and function; and this finding was interpreted as good evidence of anterior pituitary function. In Bernstein's case there was no evidence of acromegaly.

In the case herein reported there was depression of adrenal cortical activity to the extent that acute adrenal insufficiency occurred. It is believed that the well-demonstrated adrenal cortical insufficiency, the hypothyroidism, and the depression of testicular activity were all secondary to prolonged lack of stimulation by the anterior pituitary. This case bears certain similarities to the cases of Simmonds' disease reported by Sheehan (5). These cases were mainly those of Simmonds' disease following postpartum necrosis of the anterior pituitary. He pointed out that the terminal phase in Simmonds' disease is usually characterized by coma in hypoglycemia following severe anorexia, or as a result of some intercurrent illness. This case differed from Sheehan's cases chiefly in the way in which the pituitary insufficiency is believed to have developed and in the response to the therapy.

SUMMARY

The case reported is that of a 41-year-old man who showed evidence of inactive acromegaly and in whom acute adrenal insufficiency developed. The acute adrenal insufficiency responded to large doses of adrenal cortical extract and to correction of the electrolyte balance.

The patient was found to have hypogonadism, evidenced by an atrophic prostate and by testicular biopsy findings which showed atrophy of interstitial tissue and maturation arrest. Hypothyroidism was present, both clinically and as evidenced by a low basal metabolic rate.

It is believed that the polyglandular deficiency was on the basis of diminished anterior pituitary function. He was given substitution therapy and has improved remarkably. Acute adrenal insufficiency as a sequel to acromegaly is extremely rare, according to reports in the literature.

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LEYDIG (SYMPATHICOTROPIC) CELL TUMOR OF THE OVARY: REPORT OF A CASE WITH VIRILISM, INCLUDING POSTMORTEM FINDINGS

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ONE of the rarest of virilizing ovarian tumors is a tumor of the Leydig cells. These cells, which lie in the hilus of the ovary, were first described by Berger as cytologically identical with testicular Leydig cells (1). Because of their close relation to the sympathetic nerves, he called them "sympathicotropic" cells. Berger later showed that virilization could be caused by a diffuse hyperplasia or adenoma of these cells (2, 3). Only 4 cases of Leydig cell tumors with virilization have thus far been described (2, 4, 5). Another, reported by Dreyfus and Barrozo do Amaral, was obscured by the simultaneous presence of an adrenal tumor (6). Berger feels that 2 others, reported by Cosasesco *et al.* (7) and by Giordano and Haymond (8) as luteinomas, belong to the same group. In the case recently described by Waugh, Venning and McEachern, an endocrine study was made (4). In the patient reported here, an independent carcinoma of the lung confused the clinical picture and resulted in her demise. This permitted the first autopsy observation in a patient with Leydig cell tumor of the ovary.

As discussed in other reports (4, 5), there is no agreement as to terminology. Because the cells are morphologically identical with testicular Leydig cells, we shall refer to them as ovarian Leydig cells. This avoids physiologic connotation.

CASE REPORT

Mrs. C.E. (M.H. #47260), aged 47, had not menstruated for seven years. Five years before admission she noted persistent growth of hair on the beard-area of the face. One year later frontal and parietal alopecia developed. The patient otherwise remained well until June 1948 (eight months before admission), when low back pain and severe, continuous low and mid-abdominal pain developed. Three months later weight loss began and totalled 30 pounds by the time she was admitted. Four months prior to admission, the patient noted a nontender mass in the right supraclavicular area. In January, 1949, hospitalization at King's County Hospital revealed hirsutism, baldness, right pleural effusion, a blood pressure of 165/110, x-ray findings of "osteoblastic activity" in the vertebrae, normal 17-ketosteroid excretion and a pathologic report of metastatic carcinoma in a lymph node compatible with adrenal cortical origin. While in this hospital she sus-

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tained a pathologic fracture of the neck of the right humerus. In February, 1949, the patient was transferred to Montefiore Hospital.[1] System review revealed bilateral frontal headache and profound weakness of one month's duration. Nausea, vomiting and anorexia were present for three weeks and shortness of breath occurred after climbing one flight of stairs. For two years an occasional yellow vaginal discharge was noted. She had an "ulcer in stomach" at age 27 and a facial plastic operation for cosmetic reasons at age 41. Developmental history was normal.

Physical examination. The patient was a muscular, hairy, middle-aged woman who was sluggish and masculine in appearance. She could not stand without support because

FIG. 1. Appearance of patient early in hospital course. Note fronto-parietal alopecia and hirsutes of the face and chest.

of weakness. Her temperature was 98.8°, pulse 76, respirations 18 and blood pressure 136/90. Her expression was mask-like. Her face was seborrheic, moderately bearded by coarse hair (Fig. 1a), and there was marked hirsutism of the anterior and posterior chest and abdomen. Partial alopecia of the dry, normal-textured scalp hair was especially prominent in the fronto-parietal areas (Fig. 1, a and b). Coarse hair was not present in the external auditory canals. Well-healed plastic surgery scars joined both pre-auricular areas along the hair line posteriorly. She had mild scleral conjunctivitis, eccentric pupils and lid lag, but no exophthalmos. The nasal septum was slightly deviated to the left. She had a dry, beefy-red tongue with smooth edges, mild cheilosis and dry lips. Partial edentia and a greenish postnasal drip were noted. A mild anterior cervical adenopathy

[1] We are grateful to Dr. Herbert Lichtman for referring this patient and for preparing the King's County Hospital abstract.

was present. In the right supraclavicular area was a healing 3-centimeter incisional scar and two firm 8-millimeter lymph nodes. Her chest was sthenic and muscular; her breasts flat, with markedly erectile, darkly pigmented nipples. Signs of pleural effusion on the right were found. The heart was normal. Tenderness was present in the left costovertebral angle and in both lower quadrants; palpation of the latter elicited low back pain, especially on the right. The right upper quadrant was resistant but no mass was felt. A tender fracture of the neck of the right humerus was palpated. The pubic hair was sparse and the escutcheon female; the labia minora were atrophied and the clitoris enlarged, measuring 1×3.8 centimeters (Fig. 2). A white mucoid cervical dis-

FIG. 2. Note enlarged clitoris which measured 1×3.8 cm.

charge was present. Rectal examination revealed a firm, round, slightly tender mass in the cul-de-sac. There were an intention tremor of the upper extremities, hyperactive reflexes in the lower extremities and a bilaterally positive Babinski sign (no fanning on the left). The patient had a shotty nontender inguinal and axillary adenopathy and superficial varicosities of the lower extremities.

Laboratory findings. The urine was loaded with white cells, had a specific gravity of 1,020, contained protein (2 to 3 plus), and B. coli and B. pyocyaneus on culture. Blood: the hemoglobin was 14 grams, erythrocytes 4,700,000, leucocytes 9,000–13,900 with polymorphs 88 per cent, lymphocytes 8 per cent and monocytes 4 per cent. The blood serology findings were negative. The blood urea nitrogen was 20.7 and sugar 73; serum sodium 320, potassium 18, chlorides 356, calcium 11.5 and phosphorus 3.5, all in milligrams per cent. The total protein was 6.5 grams per cent, albumin 4.4 grams, globulin 2.1 grams, thymol turbidity 0, cephalin flocculation 4 plus, carbon dioxide 81 volumes per cent, alkaline phosphatase 9.8 Bodansky units and acid phosphatase 1.1 units. The

Exton-Rose glucose tolerance curve (21) showed blood sugar values of 88, 92 and 45 milligrams per cent but an intravenous glucose tolerance curve was normal. The urine showed a trace of creatine and 0.08 gram per cent or 0.648 gram of creatinine in twenty-four hours. The uric acid excretion was 0.08 gram per cent or 0.639 gram in twenty-four hours. The urea clearance was 32 and 34 per cent of the standard clearance. A cell block of fluid taken from the right chest revealed numerous tumor cells resembling carcinoma. Review of the King's County Hospital lymph-node biopsy slide showed tissue resembling adrenal carcinoma. No tumor cells were present in the spinous process marrow taken at the level of L4.

The electrocardiogram showed sinus tachycardia and the basal metabolic rate was normal.

Hormone excretions. The 17-ketosteroid excretion was 7.3 milligrams and 8.7 milligrams in twenty-four hours.[2] By Dr. E. J. Baumann's method (9), the total excretion was 2.7 milligrams, the alpha fraction 2.5 milligrams, and the beta fraction 0.16 milligram in twenty-four hours.[3] The urinary excretion of FSH was negative for 6.5, 13 and 52 mouse units in twenty-four hours.[4] Androgen and estrogen assays were not done, through mishap. The chorionic gonadotropin test gave negative results for 970 international units in twenty-four hours.

X-ray studies. X-ray films of the chest showed a pleural effusion on the right, accentuated pulmonary markings and an elongated aorta. The right humerus showed osteolytic changes through which was a fracture involving the head, neck and upper shaft. Sclerotic changes, presumably metastatic, were seen in the fifth thoracic and second and third lumbar vertebrae.Osteoblastic metastases were noted in the pelvis and left femur. An excretory urogram gave normal findings, except for a lowered position of the right kidney. Tomograms of the right renal area and entire abdomen taken during an excretory urogram revealed no adrenal masses. Except for a suggestion of some extrinsic pressure on the pylorus, a gastro-intestinal series showed nothing abnormal.

Hospital course. The gynecologist, Dr. S. Wimpfheimer, felt no ovarian mass. The patient's course went gradually downhill. She vomited frequently and shortly after admission could not retain any food. On March 24, 1949 she had a clonic convulsive seizure and vomited fecal material throughout the day. On April 3, 1949 she responded only to painful stimuli and on the following day became cyanotic, dyspneic and died.

CLINICAL PROBLEM

The patient clinically presented the adrenogenital syndrome, with muscular build, hirsutes, seborrhea, alopecia and enlarged clitoris. Etiologically, she presented a problem. It was known that metastatic cancer

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[2] We are grateful to Dr. George Escher for arranging to have one of the 17-ketosteroid determinations, the urinary FSH assay and the chorionic gonadotropin test performed at the Sloan-Kettering Institute. The ovarian hyperemia reaction described by Behnken, Lloyd and Hughes (*Am. J. Obst. & Gynec.* **56**: 930, 1948) was used to measure chorionic gonadotropin. The method described by Smith, Albright and Dodge (*J. Lab. & Clin. Med.* **28**: 1761, 1943) was used for assaying FSH.

[3] We are indebted to Dr. E. J. Baumann of the Laboratory Division of Montefiore Hospital for this determination. These results fall within the normal range for the method.

[4] Normal values vary between 6 and 52 mouse units in twenty-four hours

was present in bone (by x-ray examination), in a lymph node (by biopsy), in the pleura (by cell block) and possibly in the brain (because of seizures). The primary source was allegedly adrenal because the lymph node biopsy specimen resembled adrenal carcinoma and was reported as such by two institutions. However, there were some unusual features. The patient had normal 17-ketosteroid excretion and normal beta-fractionation in the presence of virilization. This can occur with virilizing adrenal carcinoma but is extremely rare (10, 11, 12). The possibility of carcinomatous degeneration in a hyperplastic adrenal existed, but this did not seem to be the probable explanation. We did not feel that the masculinization represented a "burnt out" process, *i.e.*, the end-results of a previously active adrenal carcinoma, because the end-organ effects persisted. Without stimulation these would return to normal. For these reasons, other diagnoses were sought.

Arrhenoblastoma was considered because of the virilization associated with normal 17-ketosteroid excretion. Although metastases to bone from arrhenoblastoma are rare, they do occur (13). Gynecologic examination of the ovaries showed nothing abnormal, however. Other virilizing tumors of the ovary such as luteoma and Leydig (sympathicotropic) cell tumors do not metastasize and are rare. Dr. Louis Leiter first suggested the possibility of an incidental carcinoma in a patient with virilism on a benign basis. The final clinical impression was arrhenoblastoma.

AUTOPSY

A mucus-producing anaplastic adenocarcinoma of the right lung was found, with metastases to the visceral and parietal pleura bilaterally, to the right lung through lymphangitic spread, and to the adrenals, spleen, mediastinum, thymus, diaphragm, ribs, lumbo-dorsal vertebrae, right supraclavicular, hilar, cervical and periaortic lymph nodes. Other findings were a pleural effusion bilaterally, atelectasis of the right lung, and focal pneumonia and bronchitis.

The remainder of the autopsy will be described in detail.

Gross

The thyroid, parathyroids, thymus, liver, spleen, pancreas, and gastro-intestinal tract were normal. The heart showed a narrow oblique probe patency of the foramen ovale.

Adrenals. The right adrenal weighed 6 Gm. and the left, 7.5 Gm. These structures were normal as to size, shape and consistency.

Genito-urinary system. No adrenal rest or tumor was found within the normal-appearing kidneys. The clitoris was enlarged (as previously described); the labia minora and vagina were moderately atrophic. Except for two small intramural fibroids, the uterus was normal.

Ovaries. Both ovaries were atrophic. The left measured 2 cm. in the greatest diameter; the right, 3 cm. Save for slight flattening of the gyrations in the right ovary, these organs were externally unremarkable. On section, the left ovary was found to be grayish and fibrotic, disclosing no evidence of oogenesis or tumor. On sectioning the right ovary,

however, a rounded tumor mass was seen, measuring 1.8 cm. in greatest diameter. The tumor occupied approximately two-thirds of the ovary and was well circumscribed but not encapsulated. Its color was a mottled, dull yellow and light to mahogany-brown. The entire urogenital line was minutely dissected for evidence of ectopic adrenal tissue. In the left broad ligament, an 8 mm. × 2 mm. perfectly formed, miniature adrenal gland was found. This ectopic rest was situated directly beneath and 3 cm. away from the left ovary. On section, it presented a bright yellow cortex and a central brown-to-gray core which could have represented either more cortical or more medullary tissue. The broad ligament on the left side was otherwise unremarkable. On the right side, however, the broad ligament disclosed the presence of numerous hydatids of Morgagni and paraovarian cysts measuring up to about 1.5–2 cm. in size. These cystic structures were filled with clear to slightly creamy opalescent fluid. Both Fallopian tubes were patent and unremarkable.

Pituitary. The pituitary was removed via the floor of the mouth, weighed 600 mg. and appeared unremarkable.

Microscopic

Thyroid. There were medium-sized acini, well filled with homogeneous colloid and lined by a single layer of flat to cuboidal epithelium. A moderate increase in interstitial fibrous tissue was noted. The *parathyroids* were unremarkable.

Thymus. This structure was atrophic. A small nest of anaplastic carcinoma was noted. Occasional tumor cells showed vacuoles and some were definite "signet ring"

FIG. 3. Microscopic appearance of the Leydig cell tumor in the right ovary. The well-circumscribed tumor occupied approximately two thirds of the ovary. H & E stain. ×9½.

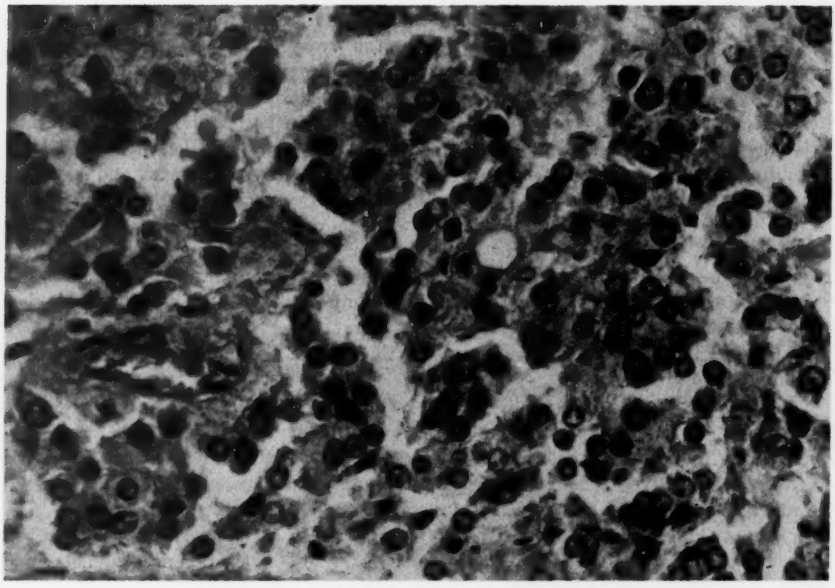

FIG. 4. Microscopic appearance of tumor. H & E stain. ×650.

elements. This section represented a transition form between the anasplastic variant and the adenocarcinoma type and indicated that there was only one *malignant* tumor.

Adrenals. The cortices, especially the glomerulosa and outer fasciculata contained moderate amounts of lipoid. There was neither atrophy nor hyperplasia of any layer. The medullae were normal. Each gland disclosed a small cortical focus of metastatic anaplastic carcinoma.

Ectopic adrenal tissue from left broad ligament. This consisted of a nodule of encapsulated adrenal cortical tissue composed of unpigmented, moderately lipoid-rich polyhedral cells. No medullary tissue was present.

Review of King's County Hospital slide of supraclavicular lymph node. This represented anaplastic carcinoma identical with that seen in the spleen. The large cells with abundant "foamy" cytoplasm, the absence of glandular differentiation and the clinical story were all factors which probably resulted in the initial diagnosis of adrenal cortical carcinoma.

Ovaries. Continuous with, and probably arising from the hilum of the right ovary, was a mass of tumor which replaced a large portion of this organ. The tumor was surrounded on all sides except that facing the hilum by a rim of compressed, atrophic and nonfunctioning ovarian tissue which was sharply demarcated from the neoplasm. The latter, however, had no capsule (Fig. 3). The tumor itself was benign and entirely different from the carcinoma described subsequently. The ovarian tumor consisted of sheets of large and often multinucleated cells with abundant granular to foamy acidophilic cytoplasm and medium to large sized, uniform, moderately chromatic round nucleoli. Varying amounts of hyaline-like stroma were noted. Mitotic figures were not seen. The tumor was indistinguishable from hyperplastic testicular Leydig cells (Fig. 4). With Masson stains, crystalloids of Reinke were seen (Fig. 5, a and b). Sudan staining revealed

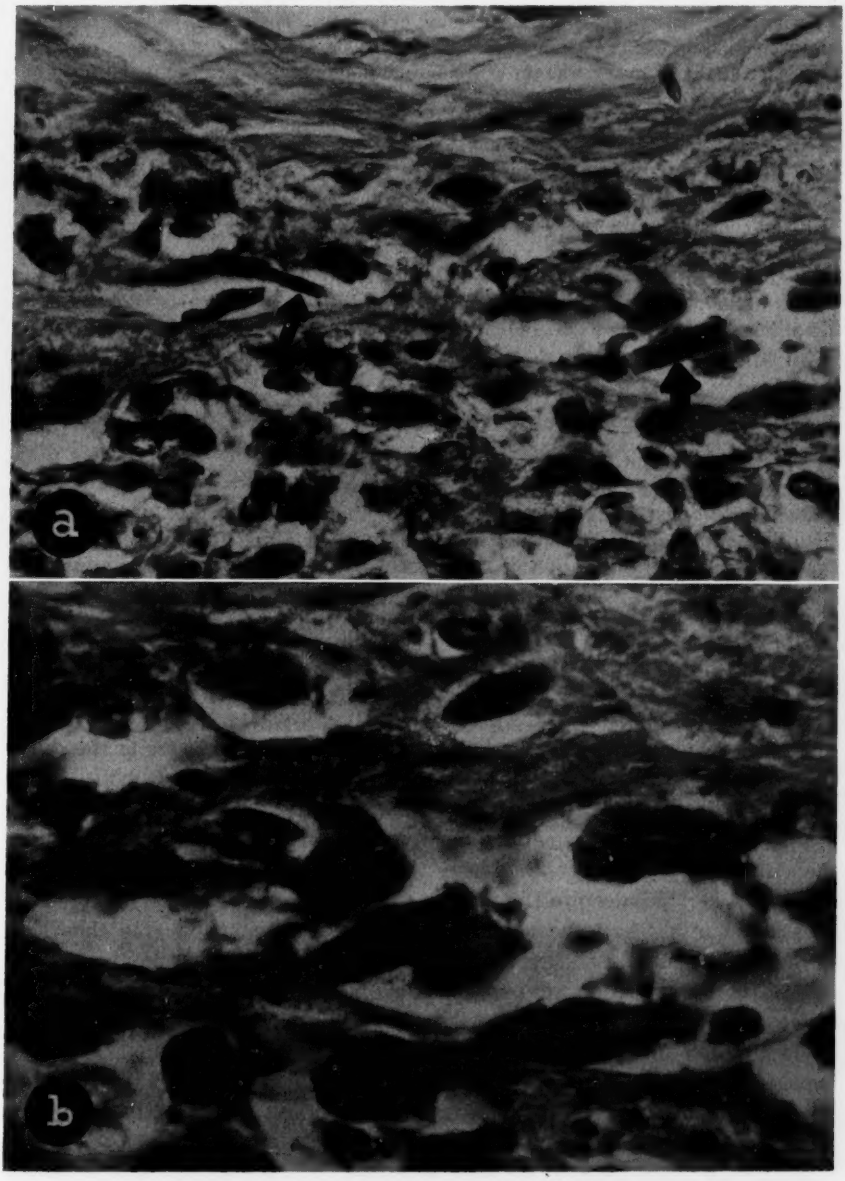

FIG. 5. a. Higher magnification of the tumor, showing characteristics of cells and crystalloids of Reinke (arrows). H & E stain. ×900.

b. Greater magnification, showing detail of crystalloid of Reinke in center of photograph. H & E stain. ×1,300.

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occasional fat particles within the cellular cytoplasm. These were doubly refractile under the polariscope. The tumor was a typical benign Leydig (sympathicotropic) cell neoplasm. The left ovary was an atrophic, nonfunctioning organ; neither tumor nor nests of Leydig cells were noted.

Right Fallopian tube. There was slight thickening of the folds of the Fallopian tube. A number of para-ovarian cystic structures filled with protein-rich fluid and lined by a single layer of cuboid or flattened epithelium were noted.

Uterus. The uterus showed marked atrophy of the endometrium, which was extremely thin and contained only a rare gland.

Breasts. The breasts showed a marked degree of atrophy.

Pituitary. There was an increase in both the acidophils and basophils at the expense of the chromophobes. The basophils were large and many were vacuolated or degranulated. There was moderate basophilic invasion of the posterior lobe. The differential count of the cells follows:

eosinophils	624
basophils	251 (41 were vacuolated)
chromophobes	135

Crooke's changes were not seen.

Lung neoplasm. The lung neoplasm was a tumor of mucous gland origin, arising from the periphery. The histologic structure varied from a well-differentiated glandular type to an anaplastic variant with transitional forms in some metastatic areas. The anaplastic variant was similar to that found in the lymph node biopsy specimen. Fat could not be demonstrated in the "foamy" anaplastic tumor cells.

DISCUSSION

The absence of obesity, purple striae, osteoporosis, hypertension (under our observation), and a disturbance in glucose tolerance made the diagnosis of Cushing's syndrome unlikely. Amenorrhea was not a helpful diagnostic feature because the patient was menopausal. However, the hirsutes, fronto-parietal alopecia, seborrhea, hypertrophied clitoris and muscular, masculine build with atrophied breasts made the diagnosis of adrenogenital syndrome certain, despite the presence of normal 17-ketosteroid excretion. The latter might have been high by the bio-assay method, had this been done. The creatinine coefficient, an index of functional muscle mass, averaged 15.2 mg. per kilogram per day. This falls within the normal range for women of 14 to 22 mg. per kilogram per day (14). Because the patient was malnourished and underweight, an abnormally high coefficient (indicating unusual muscular development) may have been obscured. Significant creatinuria above 1.0 mg. per kilogram per day, a disputable index of androgen activity, was not present.

Although some cases have been reported (10, 11, 12), it is unusual to have virilization due to adrenal cortical tumor with normal 17-ketosteroid excretion. This diagnosis was therefore discarded in favor of a virilizing ovarian tumor, despite the original lymph node biopsy report. In retro-

spect, chronic illness and starvation have been shown to lead to depression of 17-ketosteroid excretion (15, 16); this might have explained the normal excretion in our patient. Excretion of 17-ketosteroids in the 2 cases reported by Sternberg (5) was normal and in the patient reported by Waugh, Venning and McEachern (4) was moderately increased. In the latter patient, urinary glycogenic corticoids were within normal limits. Bone metastases from arrhenoblastoma have been seen; therefore, this virilizing ovarian tumor seemed to be the most likely diagnosis.

The other endocrine studies are of interest. It has been shown that ovarian Leydig cells are stimulated by chorionic gonadotropin (5). An increase in luteinizing hormone presumably could cause hypertrophy of these cells with resulting virilization. The chorionic gonadotropin test gave negative results for 970 international units in twenty-four hours. The test was made for this unitage because levels in chorionepithelioma and other tumors are very much higher than this. The urine was not tested for levels below 970 I.U. Therefore, we can only state that there was not a great increase in chorionic gonadotropin. Despite the fact that there is a marked increase in follicle-stimulating hormone at the menopause, assays of the patient's urinary FSH excretion were negative for 6.5, 13 and 52 mouse units (the normal range). A low FSH can be accounted for by increased estrogen production inhibiting its formation, by a tamponading effect of the undisclosed steroid which caused the virilism, or by primary or secondary hypopituitarism. Although estrogen assays were not made, the marked atrophy of the breasts, vagina and endometrium indicate that an overproduction of this hormone did not exist. We feel that the low (or absent) FSH can be explained by the action of the steroid which caused the virilization or by hypopituitarism secondary to wasting disease.

Histologically, the ovarian tumor in this patient resembled the other Leydig cell tumors heretofore described. Leydig cells normally occur in the ovarian hilus and mesovarium. They are almost in constant relationship to the nonmyelinated nerves and usually in the immediate vicinity of small blood vessels. Like testicular Leydig cells, there is a protean variation in form. The cells are usually polyhedral and arranged in nests or columns. The nucleus is vesicular, spherical to ovoid, and contains coarse, sparse clumps of chromatin and one to three nucleoli. The cells are occasionally multinucleated. The cytoplasm is acidophilic and granular, with varying degrees of vacuolization. Cytoplasmic inclusions include lipids, golden-brown pigment and, most characteristic of all, cystalloids of Reinke. The latter are acidophilic rod-like structures with rounded ends or rectangular ends with blunted corners. A clear zone usually surrounds the structure. They are frequently multiple in a single cell. They are indistinguishable from the crystalloids of Reinke, which are peculiar to testicular Leydig

cells. As in the case of the latter, they are found in but a small percentage of cells.

Leydig cells are prominent in the normal ovary at birth, at puberty, during pregnancy and at the menopause. These relationships plus the histologic appearance of these cells as actively secreting, suggested to Sternberg that they normally have a secretory function. He presents evidence showing that these cells respond to stimulation by chorionic gonadotropin; this also suggests functional activity. There is evidence that the ovary can elaborate male sex hormone (17–20). Both Berger and Sternberg feel that the Leydig cell may be an ovarian source of androgen.

The ovarian tumor in our patient was typical of a neoplasm of Leydig cells. The location and nuclear and cytoplasmic detail, especially the presence of the characteristic crystalloids of Reinke, established this fact. The adrenals and the adrenal rest found in the left broad ligament were normal.

Including the case reported here, 5 Leydig cell tumors have been described. All have been in menopausal or postmenopausal women. Their ages were 46 (4), 47 (this report), 50 (2), 64 (5), and 86 (5). The tumors were morphologically similar in all. Crystalloids of Reinke were found in all except Berger's case. Of the 4 cases in which the determinations were made, excretion of 17-ketosteroids was normal in 3 and moderately elevated in 1. The virilizing syndrome need not be complete, as evidenced by persistence of menses in Berger's patient and lack of hypertrophy of the clitoris in Sternberg's patient. As pointed out by Sternberg, the latter may represent a difference in end-organ response.

SUMMARY

The first autopsy observation in a patient with Leydig cell tumor of the ovary is described. The patient presented the clinical features of the adrenogenital syndrome. An unrelated carcinoma of the lung confused the clinical picture and resulted in her death. Urinary excretion of 17-ketosteroids was normal. Excretion of follicle-stimulating hormone was low or absent. The endocrine findings are discussed. Comparison is made between this patient and the 4 others previously described, in which the diagnosis was made by surgical removal of the tumor.

Acknowledgment

We are grateful to Dr. Louis Leiter, and Dr. Harry M. Zimmerman of Montefiore Hospital and to Dr. John Eager Howard of the Johns Hopkins Hospital for their advice and criticism, to Misses Norine and Emily Boetsch for preparation of the sections and to Mr. Antol Herskowitz for the photographs.

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LETTERS TO THE EDITOR

EFFECT OF ACTH AND CORTISONE ON SERUM HEXOSAMINE AND GAMMA-GLOBULIN LEVELS IN ACUTE DISSEMINATED LUPUS ERYTHEMATOSUS[1]

TO THE EDITOR:

In acute disseminated lupus erythematosus the serum hexosamine levels are abnormally elevated (Boas and Soffer, *J. Clin. Endocrinol.* **11**: 39, 1951) and the serum proteins have a characteristic pattern, namely, a low albumin, normal alpha-1 and beta-globulins and abnormally increased alpha-2 and gamma-globulin fractions (Reiner, *Proc. Soc. Exper. Biol. & Med.* **74**: 529, 1950). ACTH and cortisone consistently decrease these elevated levels of serum hexosamine and gamma-globulin. Except for increasing the albumin fraction, ACTH and cortisone effect no other changes in the remaining protein fractions. Since hexosamines are bound to serum protein, correlation studies between the two sets of simultaneously obtained data have since been performed to ascertain whether any relationship exists between the changes in hexosamine levels and in any of the protein fractions.

Serum was obtained periodically over many weeks from patients with acute disseminated lupus erythematosus who were being treated with cortisone or with ACTH. Twenty-six serum specimens from 12 patients were obtained and subjected to electrophoretic analysis and hexosamine determination. These hexosamine and protein values, simultaneously obtained, were analyzed and correlation coefficients (r) were calculated. The significances of r were determined. The only significant correlation was between hexosamine and gamma-globulin ($r = +.74$), which is significant at a probability level of less than 0.1 per cent; the slopes of the two regression lines were calculated and expressed graphically as their average (Fig. 1).

To study the problem more directly, hexosamine analyses of protein fractions separated electrophoretically were performed. The hexosamine is

[1] From the Endocrine Section of the Medical Services and the Department of Chemistry, The Mount Sinai Hospital, New York. Aided by a grant from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

expressed as per cent of protein (Gm./100 Gm.). Commercial preparations of pure normal human albumin[2] and gamma-globulin,[3] and electrophoretically separated serum proteins of one patient with lupus erythematosus were studied. The values for hexosamine in the normal human albumin fraction and in the albumin fraction from the case of lupus erythematosus

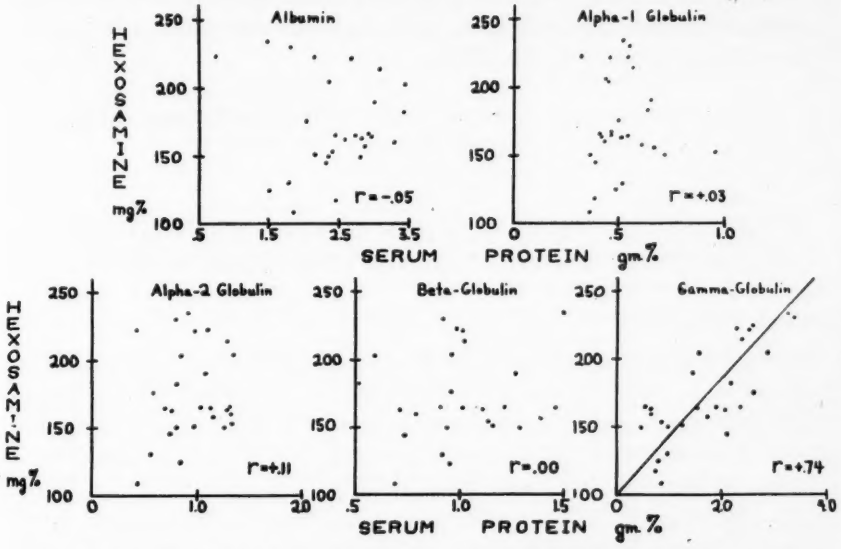

FIG. 1. Correlation data between serum hexosamine levels and electrophoretically separated protein fractions in acute disseminated lupus erythematosus.

were .34 per cent and .29 per cent respectively. The hexosamine values in the normal human gamma-globulin fraction and in the gamma-globulin fraction from lupus erythematosus were almost identical, 1.58 per cent and 1.60 per cent, respectively. In the case of lupus erythematosus the hexosamine concentration in the combined alpha-1, alpha-2 and beta fractions was 5.15 per cent (total serum hexosamine minus albumin and gamma-globulin hexosamine).

The globulins normally containing the greatest concentration of hexosamine are the alpha and beta fractions (Weimer, Mehl and Winzler, *J. Biol. Chem.* **185:** 561, 1950; Werner, *Acta physiol* **19:** 27, 1949; Werner and Odin, *Upsala läkaref. förh.* **54:** 69, 1949). Quantitatively, however, gamma-globulin is one of the largest globulin fractions. It normally contains 15 to 20 per cent of the total bound serum hexosamine. In the cases

[2] Normal serum albumin. Cutter Laboratories. Lot No. C4684.

[3] Normal serum gamma-globulin. Squibb Laboratories, Lot No. S1.

presented here, before treatment, the gamma-globulin fractions were usually increased two- to three-fold (2 to 3 grams) and could thus account in large part for the increases in total serum hexosamine. This relationship is stressed further by the positive correlation of the serum hexosamine and gamma-globulin values as they returned towards normal under the influence of ACTH and cortisone. The serum hexosamine determination in disseminated lupus erythematosus seems to be a reasonable index of the amount of circulating gamma-globulin.

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MIRIAM REINER, M.S.‡

THE PITUITARY ACTION AND ESTROGENIC EFFECT OF PARAHYDROXYPROPIOPHENONE

TO THE EDITOR:

In the February 3, 1951, issue of the Journal of the American Medical Association it was reported from Paris that a compound, parahydroxypropiophenone (paraoxypropiophenone or 4-hydroxypropiophenone) is a powerful nonestrogenic pituitary inhibitor (1). As such it was claimed to be effective in treating chorionepithelioma, toxic diffuse goiter, hyperthyroid crisis, Gilbert Dreyfus' hyperfollicullinic syndromes, Seguy's estrogenic disease, Ferrier's hypophyso-ovarian dysfunction, Rhiel's melanosis, facial melanosis, diabetes mellitus, arthritis, obesity, infertility and threatened abortion; at the same time it was claimed to be nontoxic.

Extravagant claims by such a variety of authors (2, 3, 4) bear investigation. Since each author states that the compound is nonestrogenic as well as a powerful pituitary gonadotropin inhibitor, we undertook to test these claims. Experiments were designed to test estrogenicity as well as pituitary inhibitory action. The dosage range selected, 1 to 10 mg. daily, was as high as previously selected for other compounds tested for pituitary inhibitory effects and the methods used were identical (5).

Parahydroxypropiophenone in sesame oil or in aqueous suspension was injected subcutaneously twice daily for sixteen and a half days into freshly castrated adult female Sprague-Dawley rats weighing 200 grams. The daily dose was either 1 or 10 mg. This material failed to prevent the post-castration rise in gonadotropins of the pituitaries, as judged by the ovarian and uterine weights of recipient female rats (Table 1). In contrast, .01

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mg. of estrone will prevent the postcastration rise. In reviewing the literature on the effects of parahydroxypropiophenone we noted that pituitary gonadotropin inhibition has not previously been tested directly but that pituitary inhibition has been inferred from finding "castration cells" absent following administration of the compound to castrated female rats (4).

Parahydroxypropiophenone at 1-mg. and 10-mg. daily dose levels failed to induce vaginal cornification or to maintain uterine weight following castration (Table 1).

TABLE 1. ESTROGENIC AND PITUITARY ACTIVITY OF PARAHYDROXYPROPIOPHENONE INJECTED INTO ADULT CASTRATED FEMALE RATS

	Castrated female donor rats						Gonadotropin assay —recipient rats*		
	Daily dose for 16½ days (mg.)	No. of rats	Vaginal cytology	Thymus weight (mg.)	Uterine weight (mg.)	Anterior pituitary weight (mg.)	Vaginal opening	Uterine weight (mg.)	Ovarian weight (mg.)
Paraoxypropiophenone**	1	3	Anestrus	509	103	10.7	+	89	91
	10	3	Anestrus to proestrus	471	108	9.7	+	94	98
Parahydroxypropiophenone	1	3	Anestrus	423	99	9.9	+	103	91
	10	3	Anestrus to proestrus	467	167	11.6	+	112	80
Uninjected castrated controls	—	4	Anestrus	573	117	9.3	+	93	76†
Uninjected intact controls	—	4	Cyclic	322	403	10.6	—	120	17
Uninjected intact recipient controls	—	10	———	—	—	—	—	41	15.1

* Each recipient rat received the anterior pituitary suspension of one donor rat injected twice daily for three days.

** Kindly supplied by Dr. Edward Henderson, Medical Director, Schering Corporation, Bloomfield, N. J.

† There was no significant difference between the three groups, using this assay method.

To test estrogenicity further, the uterine-weight method in the immature rat (6, 7) was applied. No estrogenic effect was noted with the 10-mg. dosage. This was at variance with Dodd's report that parahydroxypropiophenone is estrogenic at this dose level (8). It is possible that our maximal dose level was at the threshold of estrogenicity for the assay method used.

CONCLUSION

Parahydroxypropiophenone is neither a potent pituitary inhibiting agent nor an estrogenic agent in the rat.

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ENDOCRINOLOGY INDEX

The Association for the Study of Internal Secretions has undertaken to prepare a comprehensive Index of the articles published in *Endocrinology*. This Index will appear in three volumes. Volume III, covering papers published in 1940 through June, 1947, in volumes 26–40 of the journal, has been completed and is now available for distribution. Volumes I and II of the Index have not yet been completed.

Volume III of the Index is available gratis to members of the Association for the Study of Internal Secretions. The price to all non-members is $2.50. Inquiries and orders should be addressed to:

Dr. H. H. Turner, Secretary
1200 North Walker Street
Oklahoma City 3, Oklahoma.

BOOKS RECEIVED

The Diagnosis and Treatment of Adrenal Insufficiency. Second Edition. By GEORGE W. THORN, M.D., Hersey Professor of the Theory and Practice of Physic, Harvard Medical School; and Physician-in-Chief, Peter Bent Brigham Hospital, Boston, Mass., with the collaboration of PETER H. FORSHAM, M.D., Asst. in Medicine, Harvard Medical School and Peter Bent Brigham Hospital and KENDALL EMERSON, JR., M.D., Associate in Medicine, Harvard Medical School and Senior Associate in Medicine, Peter Bent Brigham Hospital; 180 pages; 25 tables; 32 figures (2 in full color), 1951. A monograph in *American Lectures in Endocrinology* edited by WILLARD O. THOMPSON, M.D., Clinical Professor of Medicine, University of Illinois College of Medicine, Chicago. Charles C Thomas, Publisher, Springfield, Illinois. Price $5.50.

Clinica ginecotocologica. Actualizaciones de temas ginecotocologicos. 1ª. Serie. Facultad de Medicina de Montevideo. Cátedra: PROFESSOR DR. MANUEL B. RODRÍGUEZ LÓPEZ; 451 pages; 5 figures. Editorial Ciencia Uruguaya, Montevideo, 1949.

Endocrinologia. By DR. RODOLFO Q. PASQUALINI, Profesor Adjunto de Clinica Médica, Director del Instituto Nacional de Endocrinologia, Buenos Aires, Argentina; 696 pages; numerous illustrations. El Ateneo, Buenos Aires, 1951.

Thyreotoxikosen und antithyreoidale Substanzen. By Prof. Dr. HANS WILHELM BANSI, APL. Professor, Hamburg University, Germany; 100 pages; 6 tables; 4 figures; 1951. Georg Thieme Verlag, Stuttgart.

La tiroides y sus enfermedades. First Spanish edition. By J. H. MEANS, M.D., Professor of Clinical Medicine, Harvard Medical School, and Chief of the Medical Services and the Thyroid Clinic, Massachusetts General Hospital, Boston, Massachusetts, with the collaboration of R. W. RAWSON, M.D., Asst. Prof. of Medicine, Harvard Medical School, and OLIVER COPE, M.D., Asst. Prof. of Surgery, Harvard Medical School; translated by Drs. CARLOS GALLI MAININI and ARTURO OÑATIVIA; 622 pages; 62 figures. Editorial Artecnica, Buenos Aires, Argentina.

Tratado de endocrinologia clinica. Volumes I and II. By DR. JUAN C. MUSSIO FOURNIER, Professor of Clinical Endocrinology, Faculty of Medicine, Director of the Institute of Endocrinology, Montevideo, Uruguay, with the collaboration of DRS. A. S. ALBRIEUX, L. B. AREY, J. C. BARSANTINI, J. BAUER, W. BUÑO, A. DE CASTRO, E. B. DEL CASTILLO, E. FELS, C. F. FLUGHMANN, L. FRANKEL, B. A. HOUSSAY, L. LAROCHE, J. J. LUSSICH SIRI, G. POUMEAU DELILLE, F. F. ROCCA, H. SELYE, E. SEVRINGHAUS, H. ZONDEK (Vol. I) and A. S. ALBRIEUX, E. M. CHAPMAN, E. HAMLIN, JR., E. J. KEPLER, W. LOCKE, G. MARAÑON, J. MORATÓ MANARO and J. J. MORTON (Vol. II); 1972 pages; 335 figures. Guillermo Kraft, Limitada, Buenos Aires, Argentina.

Treatment of the Nephrotic Syndrome. By LEE E. FARR, M.D., Physician-in-Chief, Brookhaven National Laboratory Hospital, Upton, L. I., New York; 68 pages, 1951. A monograph in *American Lectures in Circulation* edited by IRVINE H. PAGE, M.D. and ARTHUR CURTIS CORCORAN, M.D., Research Division, Cleveland Clinic Foundation, Cleveland, Ohio. Charles C Thomas, Publisher, Springfield, Illinois. Price $1.75.